PATHOLOGICAL PROCESSES IN MALARIA AND BLACKWATER FEVER



1–5 *Plasm di m ax* 1 Ring form An oebo d f rm 3 Schi ont 4 Male gametocyte 5 F male g n tocyte

6–10 *Plasm di m f k p rum* 6 Ring and *appl qi é* forms 7 Amoeboid form 8 Schizont 9 Male gametocyte 10 Femal gam tocyte

11–15 *Plasmod m malaria* 11 Young band fo m 12 Old r band form 13 Schizont 14 Mal g metocyte 15 Female g m tocyt

16–18 *Plasmod m al* 16 Ring form 17 A 18 Schizont

19 Pigmented monocyt in peripheral blood f ction

PATHOLOGICAL PROCESSES IN MALARIA AND BLACKWATER FEVER

BY

BRIAN MAEGRAITH

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To My Wife

INTRODUCTION

This book has grown out of a series of lectures given over the past two years to post-graduate students of tropical medicine Its object is to define as far as possible the basic physiological and pathological processes which determine the reaction of the animal body to invasion by the malaria parasite and the appearance of blackwater fever The discussion centres around most of the important organs affected with the exception of the lungs and the gastro intestinal tract Reference to the latter has been largely omitted because the changes occurring in them are primarily the same as those in other organs

It was hoped at one stage that it would be possible to present a coherent picture of malaria as a whole but the lack of information is too great at present to permit this Certain processes have however been found common to the development of lesions in all the organs notably generalized anoxaemia vascular endothelial damage and general and local circulatory changes which result in the production of tissue anoxia

If this book does no more than point out some of the appalling gaps in our knowledge and stimulate some intelligent research I believe it will have served a useful purpose

ACKNOWLEDGEMENTS

THE chapter on the malaria parasites has been written by my colleague Dr R H Black who served in the Land Headquarters Medical Research Unit Cairns Australia during the recent war and who has had unique experience in dealing with *in vitro* cultivation of the human malaria parasite

A great deal of the planning of this book has been facilitated by the labours of others and I should like to acknowledge my indebtedness to the following authors whose works have been freely used

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BRIAN MAEGRAITH

Liverpool

August 26 1947

PATHOLOGICAL PROCESSES IN MALARIA AND BLACKWATER FEVER

CHAPTER I

HUMAN MALARIA

GENERAL P. VIVAX MALARIA Incubation period and prodromal symptoms — The attack — The paroxysm and interval — Other signs and symptoms — Course of the disease P. MALARIAE MALARIA Incubation period and prodromal symptoms — The attack — The paroxysm and interval — Other signs and symptoms — Course of the disease UNCOMPLICATED P. FALCIPARUM MALARIA Incubation period and prodromal symptoms — The attack — Other signs and symptoms — Course of the disease PERNICIOUS ATTACKS Hyperpyrexia — Algid malaria — Cerebral malaria — Bilious remittent fever — Gastro-intestinal form DIAGNOSIS OF MALARIA CHEMOTHERAPY OF MALARIA The uncomplicated attack — Relapses — Complicated and pernicious cases — Suppression — Children BLACKWATER FEVER Distribution and aetiology — Clinical picture — Diagnosis — Treatment

THIS Chapter contains a very brief account of some of the main features of the clinical manifestations of malaria and is meant to act as an introduction to the disease for those unfamiliar with it It is not in any sense intended to be more than this Detailed descriptions of the signs and symptoms associated with pathological changes in certain organs will be found in the Chapters dealing with the various organs concerned A short note on treatment has been added for the sake of completeness *P ovale* infections are not described

Man acts as the intermediate host in the life cycle of the malarial plasmodia which infect him Infection occurs in natural circumstances as a result of parenteral introduction of sporozoites by the infective mosquito There is some evidence that infection may in rare instance be acquired across the placenta giving rise to so-called congenital malaria The bite of one mosquito may introduce sufficient sporozoites to establish the disease In such natural infections it is often assumed that the sporozoites are injected directly into the blood stream but there is no proof of this and it is apparently not essential to the establishment of the disease since Boyd and Stratman-Thomas (1934) have shown that infection can be obtained by the introduction of sporozoites into blister fluid Gordon and Lumsden (1939) watched the mouth parts of mosquitoes in the act of biting the web of a frog s foot and observed two methods of feeding one by direct insertion of

for some days until the appearance of trophozoites This silent phase has been explained on the grounds of the existence of an exo-erythrocytic tissue phase of the parasite intervening between the sporozoite and the known asexual cycle It is not possible by increasing the dose of inoculum to reduce this period to less than a certain minimal time the length of which depends on the species of Plasmodium involved By subinoculation of blood from the inoculated subject to a non-infected recipient it has been ascertained that the blood of the former is never infective before the fifth day from inoculation although it is commonly infective by the seventh to the ninth day depending on the species of Plasmodium (Raffaele 1937 Fairley 1947)

The incubation period is usually considered to end with the appearance of fever Parasites may become first detectable in the blood at the time of the appearance of fever but they are frequently present a day or two before the rise in temperature and occasionally appear later According to Ross the parasite concentration in the blood must reach a certain critical level before fever develops Boyd (1938) found this pyrogenic level was about 10 parasites per cu mm in the case of induced *P vivax* malaria The level was higher in cases in which the appearance of parasites in the peripheral blood preceded the rise in temperature It is dependent to some extent on the immune reactions of the host as can be seen particularly well in *P falciparum* infections In negroes very high parasite densities may be recorded before the temperature rises Kitchen (1941) quotes one case for instance in which 71 000 parasites per cu mm were present on the first day of the fever and in which parasites were present three days before the febrile attack Such initial high densities are uncommon in white subjects in whom the appearance of fever commonly precedes that of the parasites In both *P falciparum* and *P vivax* infections the pyrogenic level is usually higher in relapses presumably because of the development of some acquired tolerance to the parasite (Kitchen 1941)

The fever and associated signs and symptoms of an overt malarial attack usually show a periodicity which is dependent to a large extent on the growth and maturation of the parasite as it undergoes its asexual cycle The febrile paroxysm appears about the time of maximum segmentation and rupture of the mature schizonts with liberation of free merozoites into the plasma In the early stages of an attack the developing broods of parasite are often not closely synchronized and the fever is irregular but after a few days the parasites fall into step and the broods mature within a few hours of one another Synchronicity is most evident in *P vivax* infections least in *P falciparum*

the fascicle into a capillary and the other by absorption of blood from pools formed by the injury to the vessels resulting from the insertion of the fascicle These experiments indicate that the mosquito does not have to rely on direct capillary penetration and that such penetration is largely fortuitous Presumably therefore the direct injection of sporozoites into a vessel must be equally fortuitous

Artificial infection may be achieved by causing infected mosquitoes to bite suitable subjects or by the intravenous injection of suspensions of sporozoites prepared from the glands of infected mosquitoes (James Nicol and Shute 1927) Shute (1937) reported successful infection after injection of as few as fifty sporozoites

Infection may also be acquired artificially by injection of trophozoites intravenously intramuscularly or subcutaneously for therapeutic purposes Accidental infection has followed blood transfusion from infected donors or stored blood and from the use of a communal syringe e g by drug addicts (Biggam 1929 Hutton and Shute 1939 Black 1940 Sharnoff Geiger and Selzer 1945) Shortt and Menon (1940) have reported successful infection in monkeys and chickens following the oral administration of defibrinated infective blood Infection has also been established by the injection of a single trophozoite or merozoite in *P knowlesi* and *P cathemerium* malaria (Coggeshall and Eaton 1938 Stauber 1939) and Kitchen (1941) reports the transmission of *P vivax* malaria with as few as ten trophozoites The latter points out that a successful take after injection depends not only on the dose of inoculated material and its stage of development but also very much on the recipient and his state of natural or acquired immunity to the introduced plasmodia

The interval between inoculation of the infective material and the appearance of the disease (taken either as the first appearance of parasites in the peripheral blood or as the first rise of temperature to 100° F) is known as the incubation period In trophozoite induced malaria the length of this interval is roughly in inverse proportion to the dose administered It is possible with a sufficiently heavy inoculum to obtain immediate infection Boyd and Kitchen (1936) have recorded passive paroxysms in some heavily inoculated cases related to the division of the injected brood of parasites (Boyd 1941)

The incubation period of sporozoite induced malaria whether naturally or artificially acquired is usually called the intrinsic incubation period to distinguish it from the time required for parasitic development in the definitive host Shortly after injection sporozoites disappear from the blood stream which becomes free from all parasites

blood most commonly 13 days after inoculation but they may appear as early as the eighth day if the dose of sporozoites is sufficiently large Occasionally the incubation period in both natural and artificially induced *P vivax* infections may extend into months (Boyd and Katchen 1938 Shute 1946)

During the last few days of the incubation period the patient may suffer from prodromal symptoms particularly headache limb pains and backache anorexia slight nausea and even vomiting He frequently complains of shivering feelings which in relapsing cases often have the same periodicity as the paroxysms which subsequently develop The prodromal symptoms in *P vivax* malaria are however seldom severe and may be absent altogether

The attack

The incubation period terminates with the onset of the disease which is usually defined as the point at which the body temperature first rises to 100° F or above Thus does not often occur before the tenth day following infection Parasites may be detectable for the first time in the peripheral blood one or two days before or after the febrile onset

The onset may be accompanied in a relapse by a rigor and febrile paroxysm but this is unusual in the primary attack in which the fever becomes continuous or irregularly remittent for the first few days during which rigors are uncommon As the disease progresses however the regular paroxysm makes its appearance and usually by the end of the first week the pattern of intermittent febrile paroxysms separated by apyrexial and often symptom free intervals is established The periodicity of these paroxysms may vary for some time but eventually they become tertian or quotidian and usually remain so although in some cases changes in the rhythm may occur spontaneously from time to time

The paroxysm and interval

In the experience of most observers paroxysms of benign tertian malaria develop in the *post meridian* hours Katchen found this to be so in 90 per cent of his cases 70 per cent of which started the paroxysm between 3 and 9 p m (Stratman-Thomas 1941) The paroxysms develop in three well-defined stages i e the cold stage or the chill the hot or fever stage and the sweating stage

The *cold stage* is characterized by a subjective feeling of intense cold associated with a rapidly rising body temperature and various degrees

Symptoms appear as a rule only during the stages of the completion of schizogony and the liberation of merozoites Where the cycle of schizogony takes 48 hours as in *P vivax* and *P falciparum* infections the classical periodicity of fever is tertian the paroxysms occurring every third day but in these infections daily or quotidian paroxysms are also very common indicating two broods of parasites maturing on alternate days Quotidian periodicity may follow a single inoculation and cannot therefore be explained in terms of successive infections The classical periodicity in *P malariae* infections in which the plasmodia mature every 72 hours is quartan i e the paroxysms appear every fourth day In this infection however almost every conceivable variation of periodicity from quotidian to quartan has been recorded

After the short irregular interval immediately following the onset of symptoms some kind of periodicity is usually established in *P vivax* and *P malariae* and less often in *P falciparum* malaria but the periodicity is by no means fixed so that a tertian fever may change suddenly into quotidian and vice versa Such changes of periodicity may be produced artificially by various methods of treatment e g the administration of vaccine fever therapy has been shown to change quotidian periodicity into tertian (Plotner 1944) probably by eliminating one brood of parasites Periodicity may also be upset by altering the habits of the patients with regard to their periods of activity and rest (Young Coatney and Stubbs 1940)

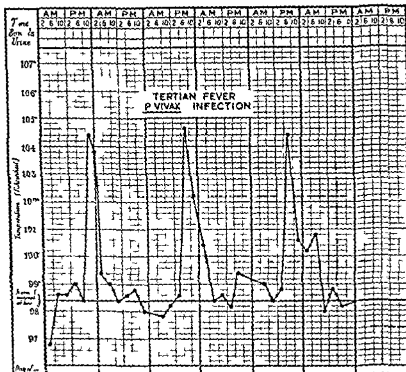
The duration of an attack of malaria depends on both the patient and the strain of parasite *P vivax* and *P malariae* infections tend to be long for instance Kitchen (1941) quotes a case of uninterrupted quartan malaria which continued for over 300 days *P falciparum* infections are usually shorter Spontaneous cure is followed in more than half the cases by return of symptoms at intervals after weeks or months or even years All three infections may lie latent for long periods The relapse follows the pattern of the primary attack in most cases except that the periodicity of the symptoms is determined from the onset and the length of the attack is usually shorter

P VIVAX MALARIA (Benign Tertian)

Incubation period and prodromal symptoms

The incubation period of benign tertian malaria varies from 10 to 17 days In artificially induced malaria the length of this period depends to some extent on the dose of sporozoites inoculated into the subject Stratman-Thomas (1941) states that parasites appear in the peripheral

stage the patient is exhausted and frequently passes into deep sleep from which he wakes considerably refreshed and subjectively much better



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The temperature remains normal or below until the start of the next paroxysm This interval is usually symptom free Occasionally the temperature in the interval may rise above normal

Other signs and symptoms

Paroxysms as explained above will repeat at regular intervals during the clinical activity of the disease In addition to the paroxysms however there are other symptoms of importance The spleen enlarges during the disease and is often palpable by the second week It enlarges slightly in some cases during the paroxysm Anaemia develops as the disease progresses and may in exceptional cases become very severe although it is usually not pronounced (see Chapter III) The

of shivering and rigor Chills are uncommon in the primary attack during the initial irregular fever but accompany the febrile paroxysms as soon as they are established In the relapse however they are present with the first paroxysms They may be present at every paroxysm or in quotidian fever may appear only in association with every other febrile attack The temperature at the start of the shivering is usually below 100° F and may have reached 104–106° F by the completion of the cold stage In the primary attack the severity of the rigor and the height of the temperature reached in the cold stage may increase for the first one or two weeks before reaching a maximum and similarly the total length of the cold phase may increase from 10–15 minutes to a maximum of 45–60 minutes as the disease progresses

The cold stage begins abruptly The patient complains of feeling bitterly cold and begins to shiver frequently passing into a violent rigor The skin is pale and may be slightly cyanotic in the extremities There may also be some apparent cyanosis of the mucous membranes The body temperature rises rapidly The pulse is small and fast although the blood pressure may rise Anorexia nausea and vomiting are common and sometimes accompanied by epigastric pain and discomfort *By the second week of the illness the spleen is usually palpable* and tender and may often appear to enlarge slightly during the paroxysm Polyuria is common in the cold stage and the urine is of low specific gravity and concentration and may contain albumin

The *hot stage* follows the cold The rigor ceases and the patient feels uncomfortably hot The skin becomes flushed hot and dry The temperature may continue to rise or remain at the level reached at the end of the cold stage The pulse is full and bounding and the blood pressure tends to fall the diastolic pressure falling proportionately faster than the systolic Respiration is rapid and there may be some unproductive coughing Nausea and vomiting are common and the patient frequently complains of thirst He is restless and may be excited and delirious and occasionally passes into light coma He is usually euphoric and disorientated and complains of severe throbbing frontal headache and pains in the limbs and back

The hot stage lasts two to six hours and is succeeded by the *sweating stage* Sweating appears first on the face usually on the sides of the forehead but rapidly becomes generalized It is commonly profuse and sweat literally pours off the patient who begins to feel better immediately *The temperature has usually begun to fall before sweating occurs* but in any case once the sweating has begun it falls rapidly reaching normal or below in two to four hours After the sweating

stage the patient is exhausted and frequently passes into deep sleep from which he wakes considerably refreshed and subjectively much better

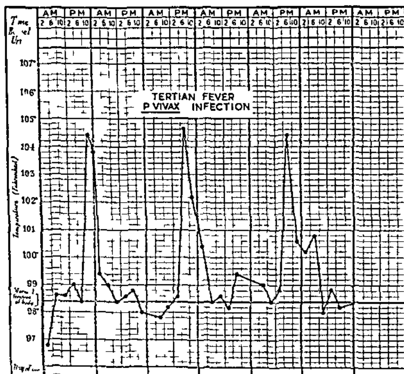


CHART 1—Teru fe er in P x inf ctio I this case yn h niz tion of maturation of p tic broods wa w ll de l ped the a ex l y l w s co plet d every 48 hours

The temperature remains normal or below until the start of the next paroxysm This interval is usually symptom-free Occasionally the temperature in the interval may rise above normal

Other signs and symptoms

Paroxysms as explained above will repeat at regular intervals during the clinical activity of the disease In addition to the paroxysms however there are other symptoms of importance The spleen enlarges during the disease and is often palpable by the second week It enlarges slightly in some cases during the paroxysm Anaemia develops as the disease progresses and may in exceptional cases become very severe although *it is usually not* pronounced (see Chapter III) The

blood picture may show macrocytes and other changes not unlike those of pernicious anaemia but the bone marrow response is essentially normoblastic and the colour index is unity or slightly less Oedema occasionally appears in the legs after the disease has lasted some weeks Kidney complications are rare but polyuria is common during the early part of paroxysm There may be a short period of oliguria in the hot stage Jaundice may appear in very severe cases but it is less common than in *P falciparum* infections *Herpes labialis* is very common In the primary attack this complication occurs after the disease is well established but in relapses it may precede by a day or two the onset of malarial symptoms It is usually confined to the mouth and lips but may spread to the nose and laterally to the ears Herpes subsides with the malarial attack

Course of the disease

A single untreated attack may last for two months or longer before it subsides Termination of the attack is indicated in most cases by reduction in the severity of the paroxysms which become irregular and finally cease and may be replaced by periodic small rises of temperature accompanied by the usual paroxysmal symptoms Kitchen (1941) considers that a symptom-less and fever-free period of about three weeks should pass before the primary attack may be considered over In about 50 per cent of cases renewed clinical activity occurs subsequent to the subsidence of an attack following a period of quiescence the length of which is determined by many factors including the strain of infecting parasite and the total duration of the infection It is customary to refer to such recurrences as relapses although James has attempted to divide them artificially into recrudescences relapses and recurrences depending upon the length of time elapsing between the termination of the primary attack and the reappearance of symptoms Relapses have been reported two years or more subsequent to the primary attack The clinical features of the relapse are indistinguishable from those of the primary attack except that the total duration of clinical activity is usually shorter in the former

P MALARIAE MALARIA (Quartan)

Incubation period and prodromal symptoms

The incubation period of quartan malaria is longer than that of benign tertian in some cases extending to 30 to 40 days between inoculation and onset

The prodromal symptoms are similar to those of benign tertian and occur during the last few days of the incubation period The patient complains of headache and vague limb pains and aches Chilly or shivering feelings are common and there may be nausea and vomiting

The attack

The clinical onset of the disease is insidious and nearly always preceded by some days by the appearance of parasites in the peripheral blood According to Boyd the parasite density of the blood at the time of the clinical onset is related to the interval elapsing between the first appearance of the parasites and the symptoms When this interval is long the parasite density is greater than when the interval is short

The acute attack of quartan malaria is usually more severe than that of benign tertian The initial attack commences with a paroxysm and regular periodicity is the rule from the start of the clinical activity of the disease Irregular remittent fever such as is seen in the initial stages of *P vi ax* infections is rare The appearance of paroxysms depends on a life cycle which is completed in 72 hours In a simple infection therefore the paroxysms occur every fourth day In naturally acquired infections such quartan paroxysms are most often seen but in artificial infections the period city of the paroxysms varies d pending on the separate development of one two or three independent broods of parasites Kitchen (1941) points out that a patient in whom three broods of plasmodia are maturing may exhibit any of seven possible arrangements of paroxysms including the simple quartan double quartan and quotidian periodicity The commonest is the simple quartan During the full period of an attack the paroxysms may conform to one type of periodicity but it is by no means uncommon particularly in trophozoite induced infection to find some degree of variation so that quartan paroxysms become double quartan and vice versa (Kitchen 1941 Craig 1909)

The paroxysm and interval

Paroxysms as a rule occur in the afternoon or late morning The time of their appearance however may vary from day to day and can be altered by readjustment of the habits of the patient (Craig 1909 Deaderick 1911 Kitchen 1941 Young Coatney and Stubbs 1940)

The paroxysms of quartan malaria are in most respects similar to those of benign tertian The three stages are clearly defined

The cold stage may be more severe or prolonged than in *P vivax* malaria On the other hand a true rigor may be absent or replaced by chilly sensations without actual shivering True rigors are most frequent in cases in which there is a quartan periodicity Both primary attack and relapse are likely to be initiated by a rigor and cold stage The patient complains of bitter cold and shivers violently There is severe headache and may be nausea and vomiting which is sometimes bilious and diarrhoea and polyuria The skin is pale or slightly cyanotic the cyanosis being most visible in the fingers and lips The pulse is rapid thin and weak and respiration fast and shallow

The temperature reached during the cold stage is usually 103-104° F *Rigors rarely precede a paroxysm in which the temperature does not* rise above 102 F

The cold stage lasts 15 to 45 minutes and is followed by a hot stage very similar to that of *P vivax* malaria but usually more severe It may last for six hours or more The patient feels hot and becomes restless The skin is flushed hot and dry The bodily temperature remains at the height attained at the end of the cold stage and may continue to rise Nausea vomiting and diarrhoea are common Respiration is rapid and sometimes accompanied by a dry cough The pulse is rapid and full and the blood pressure which may have *risen during the cold stage now begins to fall below normal* Nervous symptoms including delirium are frequently seen (Craig 1909 Kitchen 1941) There may be polyuria the urine passed being unconcentrated and of low specific gravity

The sweating stage is similar to that of benign tertian malaria Collapse is however commoner in this stage in quartan malaria and the fall of temperature more rapid and prolonged (Craig 1909 Bispham 1944) At the end of the sweating the temperature reaches a point well below normal and stays subnormal until the onset of the succeeding paroxysm The temperature may remain subnormal for days after the subsidence of an attack

Other signs and symptoms

The spleen enlarges during the attack and eventually becomes palpable It increases in size more slowly than in other forms of malaria and does not reach the dimensions found in benign tertian or malignant tertian malaria except in long-standing chronic infections or reinfections in children The anaemia of quartan malaria is as a

rule less severe than that of benign tertian and develops more slowly possibly because of the relatively slight invasive properties of the plasmodia (see Chapter III)

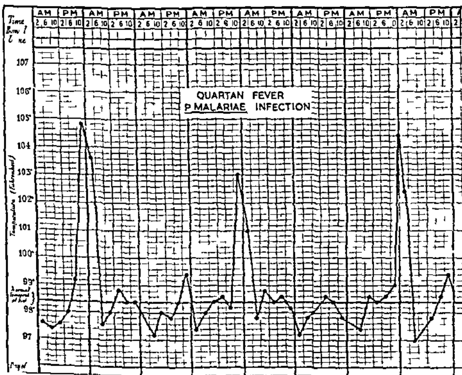


CHART 2—Q rt n f e n P f n t ton W ll d f ed h t n t t n of p tes th asex l cycle w npl t d y 72 ho rs

It has the same features as the anaemia of benign tertian the colour index being unity or slightly less and the variations in the haemoglobin concentration and erythrocytic count being parallel Jaundice is rare but liver dysfunction may be indicated by the results of various tests Oedema is more common than in benign tertian and may be associated with ascites Boyd and Proske (1941) found in two cases that the development of oedema was accompanied by a considerable depression in serum albumin content together with the passage of albumin in the urine (see Chapter IV) The latter is seen to some degree in most quartan patients and may be severe and associated with oedema and

clinical signs of an acute nephrosis especially in children (James 1910 Giglioli 1930 see Chapter VII)

Herpes labialis appears as a concomitant of quartan malaria as frequently as in other forms of the disease

Course of the disease

Quartan malaria is the most persistent and chronic of the human infections Kitchen found in his series of cases that spontaneous termination occurred in 19 to 169 days in induced quartan malaria in white patients and was of somewhat shorter duration in negroes Secondary attacks have been observed more than two years after the initial attack Relapses are common although Kitchen (1941) reported that in his series of cases they did not occur if a quiet period of more than 53 days followed the primary attack Spontaneous cure is likely but fatal cases occur Pernicious symptoms are rare and the degree of parasitaemia is usually low but Kitchen (1939) has reported a case in which the parasitic density of the blood continued to increase to death reaching 70 000 parasites per cu mm the usual maximum being about 20 000 The disease readily responds to treatment so far as the immediate symptoms of the attack are concerned but parasites may remain for long period in the blood stream without giving rise to symptoms

UNCOMPLICATED P FALCIPARUM MALARIA (Malignant Tertian)

Malignant tertian is the most serious form of human malaria It differs from the others in being caused by a species of Plasmodium which has potentially unlimited invasive powers as far as red cells are concerned and which sporulates almost entirely in the vessels of the internal organs and not in the general blood stream It is liable at any stage to develop serious and often fatal complications

Incubation period and prodromal symptoms

The incubation period is short clinical activity appearing 8 to 12 days after inoculation of sporozoites The prodromal symptoms make their appearance three or four days before the onset and are often severe The patient is tired depressed and complains of headache lumbar backache and vague aches and pains in the limbs He loses his appetite and may suffer from nausea Sometimes there may be slight daily elevations of temperature The onset itself is usually abrupt

In about a third of cases it starts with a rigor Occasionally severe or fatal complications may initiate the attack A relatively high parasitaemia may develop before symptoms appear



Fig 1—Smear f om pl t showi g por lat on of *P f lc par m*

The attack

The fever commences with associated shivering or chilly feelings but definite rigor is often absent and when present is not usually as severe as those of benign tertian or quartan malaria Nausea and vomiting are common from the onset and are frequently accompanied by severe epigastric pain

Parasites may be present in the peripheral blood for several days before the onset of clinical symptoms The degree of parasitaemia reached in this stage is higher in negroes than in white races and in the former it is unusual for the clinical activity to begin before parasites can be detected in the blood whereas in the latter the reverse is sometimes the case and high parasite densities without symptoms are uncommon (Kitchen 1941) In the untreated case the parasitaemia increases as the disease progresses but in some cases there can be detected a daily alternation of parasite density which is roughly inversely related to the appearance of the paroxysm (Kitchen 1941 see Chapter III) In the uncomplicated case except where a very heavy parasitaemia obtains it is unusual to observe parasite forms other than trophozoites and gametocytes in the peripheral blood Other forms including sporulating mature schizonts can be demonstrated in organ smears

The fever of malignant tertian malaria is often very irregular but some degree of synchronicity can usually be observed Occasionally paroxysms occur with true tertian or quotidian periodicity but this is uncommon The fever may be continuous remittent or intermittent Sometimes there are double peaks in the temperature chart resembling those of kala-azar In some severe attacks the fever may never be high and may not appear until the development of heavy parasitaemia The irregularity of the fever in *P falciparum* malaria is probably due to the successive and irregular maturation of separate broods of the parasite (Kitchen 1941 Nelson-Jones 1944 Craig 1909)

When synchronicity exists the clinical picture of the disease closely resembles that of *P vivax* infections The febrile course of the paroxysm is however usually prolonged and in cases exhibiting quotidian periodicity the successive daily paroxysms may overlap to some extent so that normal temperatures are not reached in the interval (The patient often does not appear to be very ill but the apparent benign course of the disease is deceptive because serious complications may arise at any stage either in the initial attack or subsequent relapse)

Other signs and symptoms

Considerable anaemia is liable to develop in the acute attack *P falciparum* attacks all ages of red cells impartially so that high degrees of parasitaemia may be reached very rapidly with great concomitant destruction of the invaded cells and probably also of the uninvaded cells A severe case may in a week from the onset lose as much as two million red cells per cu mm Lytic crises of great severity occasionally occur (usually associated with the passage of haemoglobin in the urine) in which the erythrocyte count may fall to one million or fewer cells per cu mm in the course of 24 hours The anaemia is commonly associated with a mild leucopenia characterized chiefly by an absolute reduction in granulocytes and an increase in large mononuclear cells

The spleen enlarges in all cases and is usually palpable and frequently tender During paroxysms the splenic size sometimes increases decreasing again in the interval The ultimate size attained by the organ in the acute attack is smaller than that reached in *P vivax* infections probably because they are of short duration After repeated attacks of malignant tertian and in chronic infections especially in children the spleen may become enormous

Enlargement and tenderness of the liver is not uncommon in severe malignant tertian malaria associated with epigastric distress and bilious vomiting and jaundice

Herpes labialis is a common accompaniment of malignant tertian and behaves in the same way as it does in benign tertian malaria disappearing subsequent to the control of the malaria infection

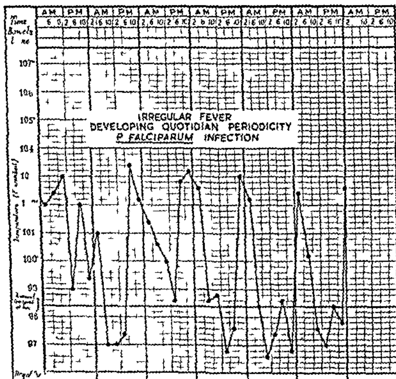


CHART 3—Irregular fever at onset of clinical activity of *P falciparum* infection. Quotidian periodicity is becoming established probably due to presence of two broods of parasites maturing every 48 hours the asexual cycles being completed on alternate days

Course of the disease

The acute attack of *P falciparum* malaria is shorter in duration than either benign tertian or quartan If adequately treated and if no pernicious symptoms develop there is little hazard to life The disease relapses but renewal of clinical activity is uncommon after a year from the initial attack In chronic cases or in subjects constantly exposed to fresh infections or a series of acute attacks severe anaemia and splenomegaly develop and the patient may go on to the state of malarial cachexia (James 19.. Hehir 19.7 Kitchen 1941 Bispham

The fever of malignant tertian malaria is often very irregular but some degree of synchronicity can usually be observed Occasionally paroxysms occur with true tertian or quotidian periodicity but this is uncommon The fever may be continuous remittent or intermittent Sometimes there are double peaks in the temperature chart resembling those of kala-azar In some severe attacks the fever may never be high and may not appear until the development of heavy parasitaemia The irregularity of the fever in *P falciparum* malaria is probably due to the successive and irregular maturation of separate broods of the parasite (Kitchen 1941 Nelson-Jones 1944 Craig 1909)

When synchronicity exists the clinical picture of the disease closely resembles that of *P vivax* infections The febrile course of the paroxysm is however usually prolonged and in cases exhibiting quotidian periodicity the successive daily paroxysms may overlap to some extent so that normal temperatures are not reached in the interval (The patient often does not appear to be very ill but the apparent benign course of the disease is deceptive because serious complications may arise at any stage either in the initial attack or subsequent relapse)

Other signs and symptoms

Considerable anaemia is liable to develop in the acute attack *P falciparum* attacks all ages of red cells impartially so that high degrees of parasitaemia may be reached very rapidly with great concomitant destruction of the invaded cells and probably also of the uninvaded cells A severe case may in a week from the onset lose as much as two million red cells per cu mm Lytic crises of great severity occasionally occur (usually associated with the passage of haemoglobin in the urine) in which the erythrocyte count may fall to one million or fewer cells per cu mm. in the course of 24 hours The anaemia is commonly associated with a mild leucopenia characterized chiefly by an absolute reduction in granulocytes and an increase in large mononuclear cells

The spleen enlarges in all cases and is usually palpable and frequently tender During paroxysms the splenic size sometimes increases decreasing again in the interval. The ultimate size attained by the organ in the acute attack is smaller than that reached in *P vivax* infections probably because they are of short duration After repeated attacks of malignant tertian and in chronic infections especially in children the spleen may become enormous

Enlargement and tenderness of the liver is not uncommon in severe malignant tertian malaria associated with epigastric distress and bilious vomiting and jaundice

Herpes labialis is a common accompaniment of malignant tertian and behaves in the same way as it does in benign tertian malaria disappearing subsequent to the control of the malaria infection

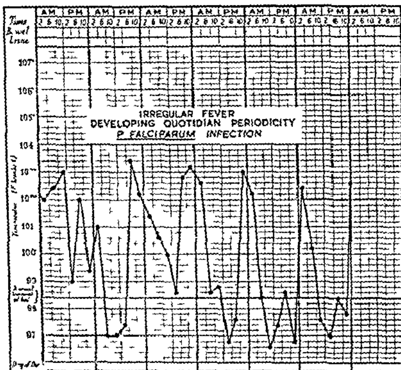


CHART 3—Irr gular f er t c n men ment of linical activity of *P f l par m* inf ction Q tidian period ty is bec ming est bli h d p bably due to p sen of tw b ood of p asites m turing e ery 48 h urs th asexual y les being mpl ted on lterm t das

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1944) Although malarial infection is the underlying aetiological factor in this latter condition the picture is almost always complicated by ill nourishment and dietary deficiencies so that it becomes impossible to distinguish the effects of the disease *per se* from those grafted on to it by lack of protein essential substances and so forth

PERNICIOUS ATTACKS

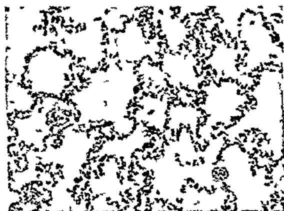
Pernicious forms of malaria are serious variations and complications of the disease produced by the same parasites as the common milder infections They should therefore be regarded not as separate disease entities but as syndromes arising during the clinical course of malaria They can be divided roughly into syndromes of a general type and those which indicate specific attacks on various organs Thus on the one hand there may be an overwhelming infection of the kind seen in *P knowlesi* malaria in monkeys or the appearance of a state of complete vascular collapse and on the other syndromes apparently almost exclusively involving the central nervous system or the gastro-intestinal tract Pernicious attacks may develop at any stage of the disease The onset of a primary attack may be accompanied by pernicious symptoms or they may appear during an apparently mild attack or in a relapse On the whole however they occur most frequently in cases which have suffered from repeated attacks particularly if these attacks have been inadequately treated (Craig 1909) A history of irregular and inadequate suppressive therapy is also sometimes associated with pernicious attacks As a rule in pernicious malaria there is a high parasitaemia but this is not always the case as the parasites may be concentrated in the organs and not appear in great numbers in the peripheral blood stream

Kitchen (1941) gives a list of over forty syndromes and symptoms of pernicious malaria grouped under the various bodily systems they involve namely nervous system gastro-intestinal system cardiovascular and haemopoietic systems respiratory system genito-urinary system and others It would be tedious to attempt to deal with any but the commoner types and we shall discuss here only a few of the more important syndromes

Hyperpyrexia

This manifestation of pernicious malaria shows itself as a rule during the course of an ordinary attack of malignant tertian malaria It may on the other hand complicate the picture of other forms of pernicious

attack particularly the cerebral type The characteristic feature is a rapid rise of bodily temperature to 107° F or higher The rise of



Fɪɢ 2—Sect n of lung fr n ase of mal g a t t uan n al a N t o d m fluid in lveoli and a cumulatio t f p a tized eryth ocyt an the s pt l p ll e

temperature which occurs in a matter of a few hours continues in most cases until death unless suitable treatment is instituted The patient is dyspnoeic and becomes comatose The skin is hot dry and may be slightly cyanotic The dryness of the skin is important since there appears to be some inhibition of sweating (This may lead in hot climates to the incorrect diagnosis of heat hyperpyrexia)

Algid malaria

The patient passes rapidly into a condition of complete collapse and muscular asthenia resembling surgical shock His appearance is characteristic (Paisseau and Lemaire 1916) The expression is anxious the face drawn and pinched and the eyes sunken The breathing is shallow and the pulse thin and fast and easily compressible The skin is pale or slightly cyanotic feels cold and is covered with clammy sweat Although the skin feels cold the rectal temperature is often raised above normal reaching 101 to 103 F There is frequently intense abdominal pain centred mainly in the epigastrium and sometimes accompanied by persistent vomiting or profuse watery diarrhoea The blood pressure is low Parasitized erythrocytes are usually present in large numbers in the peripheral blood In untreated cases and often in treated cases the issue is fatal the patient passing into coma with irregular thready pulse and rapid irregular breathing

and dying from peripheral circulatory failure The relation between this syndrome and that of acute adrenal insufficiency is discussed in Chapter XI

Cerebral malaria

A malarial attack concentrated mainly on the brain may appear clinically in many forms all of which are usually classified as cerebral malaria The predominating clinical features may be those of coma delirium meningitis and so on

The onset may be sudden but is more commonly gradual developing in the course of an apparently uncomplicated case of malaria In the acute case with sudden onset coma appears without warning and death ensues rapidly The more slowly developing case of cerebral malaria is often first detected by the odd behaviour of the patient who may become confused and pass into acute delirium with hallucinations accompanied by violence More commonly the patient complains of increasing headache and becomes drowsy or restless and depressed He continues into coma slowly and progressively In the comatose state he lies deeply unconscious and quiet except for irregular muscular twitchings and tremors The face is pale or suffused The pupils are contracted sometimes unequally so There may be paralysis of the external orbital muscles causing squint The deep reflexes may be abolished or sometimes exaggerated and the Babinski sign is sometimes positive There may be stiffness of the neck and muscle twitchings going on especially in children to convulsions which may be epileptic in type Hemiplegia may also develop The pulse in the early stages is full and bounding but as the case progresses towards death it becomes small fast and thready The respirations are fast and shallow ultimately there may be stertorous and even Cheyne-Stokes breathing The skin is hot and dry to begin with but in the stage of final collapse it is pale and covered with fine cold sweat The temperature is usually in the region of 101 to 103° F but it may be subnormal and in some cases becomes raised uncontrollably the patient passing into hyperpyrexia The cerebrospinal fluid is usually under increased pressure and contains excess of protein Any degree of parasitaemia may be present in the peripheral blood

In most cases the condition is progressive and death is the outcome unless prompt and adequate treatment is initiated early Terminal incontinence of urine and faeces is common There may be short remissions of the coma even in the worst cases the patient becoming temporarily lucid for a brief interval before lapsing back into coma

which is usually fatal In recovery there may be evidence of central nervous involvement especially persistent headache and sometimes paresis of groups of muscles

Bilious remittent fever

This complication of malaria is one in which the patient usually suffers from abdominal discomfort from the outset There is severe epigastric pain nausea develops early and vomiting follows becoming severe and persistent The vomit contains bile sometimes coffee grounds and even unchanged blood Diarrhoea is common and usually severe with the passage of copious watery stools sometimes containing blood The patient passes rapidly into a state of dehydration accompanied as a rule by a low delirium The spleen is palpable and tender and the liver enlarges appreciably and is also tender The skin becomes icteric as a rule by the second day of the disease Jaundice increases rapidly Epistaxis is frequent The urine is scanty and contains bile pigment The red cell count falls rapidly and a high degree of parasitaemia is usual In the beginning of the attack the temperature may show some periodicity but it characteristically becomes remittent and may closely resemble the fever of typhoid The outcome is usually fatal

In the form of bilious remittent fever described above the main malarial attack is on the liver Fairley (1933) described another form in which the jaundice is of a different type and in which there is no bilirubin in the urine and the van den Bergh reaction is indirect or biphasic and evidence of liver damage is not so obvious

Gastro-intestinal forms

Dysenteric pernicious malaria is characterized by the passage of frequent stools consisting mainly of blood mucus and epithelial and cellular debris There is tenesmus colicky abdominal pain and tenderness of the abdomen particularly along the line of the colon There may be nausea and vomiting Fever is usually high and remittent The condition is practically indistinguishable from acute bacillary dysentery and must be differentiated from it by examination of the peripheral blood and the blood of the stool both of which will be found to contain plasmodia Stool culture is of course essential

Choleraic pernicious malaria may very closely resemble true cholera The stools are frequent profuse and watery and may contain mucus blood and bile pigments The loss of fluid from diarrhoea leads to

dehydration and collapse associated frequently with severe abdominal cramps nausea and vomiting which may be bilious The pulse is fast

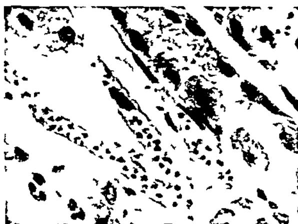


FIG 3—Section of stomach in case of malignant tertian malaria showing intense accumulation of parasitized erythrocytes in capillaries

and small and the blood pressure falls and the skin becomes cold and clammy The temperature is usually raised above normal Oliguria is common and may go on as in true cholera to complete suppression followed by death in uraemia The general picture in the final stages resembles that of algid malaria

DIAGNOSIS OF MALARIA

Malaria can be diagnosed for certain only by the identification of the parasite Normally this will be found in the peripheral blood but occasionally it may be necessary to search for it in the sternal marrow The chances of finding it in the marrow when it cannot be discovered in the blood are very small Failure to find the parasite on one or two occasions does not exclude the diagnosis The probable degree of parasitaemia cannot be estimated clinically

For details of methods of examination and identification of parasites see Chapter II Clinical diagnosis which is of importance only when laboratory methods are not available is discussed in the standard text-books (Stitt 1942 Manson-Bahr 1945)

CHEMOTHERAPY OF MALARIA

Specific treatment with antimalarial drugs should be started immediately diagnosis has been confirmed Oral administration should

be aimed at but it may be necessary to use parenteral routes in special cases e g intractable vomiting vascular collapse (for example in algid malaria) coma hyperpyrexia and hyperparasitaemia

Quinine mepacrine and paludrine are now in general use for therapeutic purposes Plasmoquine (pamaquin) is not used alone but only in combination with one of the other drugs It is never given parenterally Certain sulphonamides e g sulphadiazine have also been administered with some degree of success The point of action of antimalarial compounds in relation to the parasites is discussed in Chapter II Details of treatment should be sought in appropriate textbooks

The uncomplicated attack

Uncomplicated cases of all forms of human malarial infection will respond to any of the following courses of treatment (Maegraith 1946 Maegraith *et al* 1946)

(*a*) Quinine (dihydrochloride or sulphate) grains 10 t d s for 2 days
followed by grains 10 b i d for 5 days

(*b*) Mepacrine 300 mgm t d s for 1 to 2 days
Mepacrine 200 mgm t d s for the next day
Mepacrine 100 mgm t d s for the succeeding 5 days

(*c*) Quinine salts grains 10 t d s for 3 days
Mepacrine 100 mgm t d s for 5 days
Interval of 2 days (May be omitted)
Pamaquin 10 mgm b i d for 3 days

(*d*) i For Benign Tertian and Quartan malaria
On diagnosis Single dose of 300 mgm paludrine Followed by 100 mgm Paludrine twice weekly for 6 months
ii For Malignant Tertian malaria
Paludrine 300 mgm b i d for 7 to 14 days (In severe cases 500 mgm b i d may be given for the first 3 days)

Relapses

1 *Benign Tertian and Quartan malaria*

(*a*) Quinine grains 10 t d s
Pamaquin 10 mgm t d s
Drugs given concurrently for 10 days
(This may be modified so that it is given for 7 days followed by a 7-day interval after which a further 7-day course is given)

(*b*) Paludrine as above (d i)

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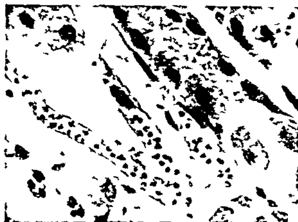


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(*b*) Paludrine as above (d i)

ii *Malignant Tertian malaria*

A full course of either mepacrine or paludrine should sterilize the infection

Complicated and pernicious cases

i Quinine dihydrochloride grains 7½ to 15 given intravenously well diluted. Intramuscular injection may be given where the intravenous route is difficult

ii Paludrine 75 to 100 mgm intravenously

iii Atebrin (mepacrine) musonate or hydrochloride 300 mgm intramuscularly never intravenously

These drugs must be given with careful aseptic precautions If given slowly they are perfectly safe and may be repeated in 6 to 12 hours Oral administration should begin as soon as possible

Suppressive dosage

i Mepacrine 100 mgm daily

ii Paludrine 100 mgm. twice or thrice weekly in subjects not previously exposed In individuals who have suffered from malignant tertian malaria it is probably better to give a full therapeutic course (d ii) followed by 100 mgm thrice weekly or once daily

Children

i Quinine One twentieth of adult dose multiplied by the age of the child in years

ii Mepacrine Not more than 150 mgm in the day up to the age of 2 years 300 mgm at 10

iii Paludrine Up to 25 mgm in the 24 hours can be given safely to children of any age

BLACKWATER FEVER

Some authors regard blackwater fever as one of the pernicious complications of malaria but it is probably better at this stage to treat the two clinical conditions separately since the precise nature of the relationship between them has not yet been determined

Distribution and aetiology

Blackwater fever is not found in regions where malaria does not exist except in individuals previously infected elsewhere Its relation to

malaria has often been emphasized (Marchiafava and Bignami 1900 Ross 1932 Foy and Kondi 1937 etc) It is seen mostly in hyper-endemic malignant tertian areas and has been reported in induced *P falciparum* infections in syphilitics (James Nicol and Shute 1932 Kitchen and Sadler 1945) In endemic areas it appears most commonly in the non-indigenous population but it may develop occasionally in the native In the former it occurs as a rule in individuals who have been in the endemic area at least a few months especially if the area is one in which constant re-infection is likely (Ross 1932) For instance Findlay and Markson (1947) in British West Africa found the liability to blackwater fever in British troops was not pronounced until after they had been at least 9 months in an endemic area On the other hand in Africans blackwater fever occurred in children or in adults who were in the habit of using suppressive drugs and mosquito nets for protection against malaria In most cases it is possible to trace a history of a succession of previous malarial attacks often inadequately treated In some however this history is absent and blackwater may appear within a few weeks of arrival in the endemic area

Plasmodia are found in the peripheral blood in about half the cases In the majority of these the invading organism is *P falciparum* but it may be *P vivax* or *P malariae* In the case described by Kitchen and Sadler of haemoglobinuria arising in artificially induced malaria all three plasmodia had at some time been inoculated into the patient but at the time of onset of haemoglobinuria only *P falciparum* was active

The pathogenesis of the haemolysis in blackwater fever is still obscure The role of antimalarial drugs is uncertain although there is considerable evidence incriminating quinine Mepacrine has been considered an aetiological factor by some but this has not been the experience of most observers (Zylmann 1944 Findlay and Stevenson 1944) Attention has been given also to the possibility of the involvement of immunity and immune reactions in the production of haemolysis Recently Gear (1946) has suggested that the invaded red cell behaves as an autoantigen and produces an antibody which acts as an autolysin in the presence of complement causing haemolysis Findlay and Markson (1947) have brought forward experimental evidence supporting the general contention that sensitization to the malaria parasite is an important factor in the genesis of blackwater fever They report a considerable increase in the incidence of blackwater fever in African troops over the years 1941–45 During this period civilian figures for the same areas showed no change The chief

difference between the living conditions of the soldiers and civilians was that the former lived in quarters where the chance of infection was low so that their immunity to local strains of parasite was reduced In this state re-infection led to haemolysis

Clinical picture

The basic phenomenon in blackwater fever is a rapid severe lysis of erythrocytes associated with the passage of haemoglobin or its derivatives in the urine

The patient usually gives a history of a series of malarial attacks usually malignant tertian prior to the onset of haemoglobinuria in the intervals between which there was persistent headache and backache accompanied by general *malaise* Occasionally a previous history of blackwater fever may be elicited

The case may present as a straightforward uncomplicated malignant tertian infection or it may commence suddenly without signs of overt malaria The onset of the haemoglobinuria may take place without immediate accompanying symptoms but in most cases it is sudden and associated with a rigor The latter is often severe and is accompanied by a rapidly rising temperature which may reach 103 to 105° F After a brief hot stage the temperature may fall to normal or below and a series of paroxysms may supervene but it is more common for the temperature to remain elevated and remittent Sometimes in fatal cases the temperature may continue rising and the patient enters a hyperpyrexial phase In some cases often severe there may be no fever throughout the illness Sweating is a common feature and appears irregularly in relation to the fever It may at times be profuse It is common for the first specimen of haemoglobinuric urine to be passed during or immediately succeeding the initial rigor Sometimes the announcement that black water has been passed may be made quite casually by the patient the passage of haemoglobin pigments usually gives rise to no symptoms at micturition and may consequently be missed especially at night It is thus often impossible to determine the true time of the onset of the condition

The patient is usually considerably prostrated from the first and is restless and anxious but fully conscious until late in the illness when he frequently passes into delirium and sometimes deep coma very similar to that of cerebral malaria In the terminal stages he is often incontinent of faeces and urine Various complications are liable to develop and are discussed briefly below

Parasites nearly always *P falciparum* may be found in the early

stages of the illness or they may be present before the onset of haemoglobinuria but once the latter commences the parasites usually disappear from the blood Their absence is therefore not of great diagnostic significance

The haemolysis may occur only once during the course of the attack or may recur spasmodically for periods varying from a few hours to several days in length with non lytic intervals of hours or days between In the haemolytic phase the destruction of erythrocytes may be extremely rapid several million cells per cu mm being destroyed in the course of 24 hours Erythrocyte counts of under a million cells per cu mm are not uncommon in severe cases sometimes after the first haemolytic crisis Occasionally the initial haemolysis is continuous and overwhelming and leads if uncontrolled to the death of the patient On the other hand mild cases occur in which the haemolysis never becomes extensive and the illness amounts to little more than the passage of haemoglobin or its derivatives in the urine

During haemolytic activity the urine contains oxyhaemoglobin and methaemoglobin in varying proportions The first specimen passed containing the pigments is usually bright red or reddish brown depending on which pigment predominates the oxyhaemoglobin being bright red the methaemoglobin dark brown Sometimes the pigments appear in the urine more gradually the urine darkening over the course of some hours In most cases after a few hours to several days the urine lightens in colour and finally becomes clear of pigment During succeeding periods of haemolysis the pigments return to the urine

In addition to the pigments the urine passed during the lytic phase contains a heavy brown amorphous deposit containing granular casts and cellular debris and is loaded with albumin In the interlytic periods the urine is clear contains little deposit and few casts and is nearly always free from albumin The reaction of the urine may be acid alkaline or neutral even during severe oliguria (Maegraith and Havard 1944 Maegraith 1944)

Great attention must be paid to the urine in a case of blackwater fever since at any stage independent of the passage of haemoglobin pigments renal failure may develop Such failure is indicated by a reduction in urinary output which may go on to complete anuria in the course of a few hours Occasionally anuria may appear without any preliminary oliguria Once anuria has developed recovery is uncommon and the patient dies in a state of progressive uraemia with rising blood urea nitrogen It may be noted here that the blood urea

nitrogen is raised to some extent in all cases of blackwater fever whether complicated by obvious renal failure or not In cases recovering from anuria the urea nitrogen returns only slowly to normal The urine passed in oliguria may or may not contain blood pigments and albumin If the oliguria develops in the post-lytic phase the urine is clear and unconcentrated Its reaction in oliguria and during suppression which is not absolute (sometimes an ounce or less may be passed in 24 hours) is not as is frequently stated always acid but may be neutral or alkaline After recovery from anuria there may be polyuria associated with the passage of large quantities of dilute urine which slowly regains its normal concentration

In many severe cases and sometimes after an apparently mild lytic phase the patient collapses and passes into a state resembling medical shock (Atchley 1930) The facies are anxious and drawn the eyes sunken The patient is restless and anxious The blood pressure is low, except in cases in which uraemic manifestations have developed following renal failure in which case the blood pressure rises as the uraemia progresses Even in these cases however in the final stages the blood pressure frequently falls rapidly especially the diastolic pressure Death occurs from vascular collapse

Interference with liver function appears early in many cases The liver swells and becomes palpable and tender Acute epigastric discomfort is common and there is nausea and vomiting which is often bilious and may become intractable Uncontrollable hiccough develops in the worst cases Jaundice appears as a rule within two days of the onset of haemolysis and deepens quickly It may or may not be accompanied by bile pigment in the urine

Reduction in plasma alkali reserve has often been reported in blackwater fever and is usually interpreted as demonstrating presumptive acidosis There are however no reliable records of changes in blood pH

The course of a case of blackwater fever may be mild or fulminating Most cases are severe All must be considered grave since at any stage fatal complications may appear such as overwhelming haemolysis total renal failure and sudden cardiovascular collapse

The death rate varies from 20 to 30 per cent Renal failure accounts for one-half of the deaths (Stephens 1937)

Diagnosis

Haemoglobinuria associated with progressive anaemia occurring in a subject who has been persistently exposed to malarial infection is

almost certainly blackwater fever In Africans the presence of sickle-cell disease must be excluded Other causes of haemoglobinuria must also be excluded particularly in relation to quinine and pamaquin The presence of haemoglobin or its derivatives should be confirmed spectroscopically where possible and must be clearly differentiated from bile pigment The absence of malaria parasites from the peripheral blood is not significant as has been already pointed out The *diagnosis of clinical complications is of the utmost importance* The volume of every specimen of urine passed should for instance be measured and recorded since anuria may develop suddenly

The rapid haemolysis and renal failure are the most striking phenomena seen in blackwater fever The true nature of the haemolysis in blackwater fever is still to be determined No specific haemolysin has ever been identified and no change in the saline fragility of the erythrocytes consistently observed Foy and his colleagues (1943) have found that the cells are in some cases abnormally fragile in a lysolecithin system and believe that the fundamental factor at work in the haemolysis is extracellular and is capable of lysing normal red cells and rendering the cells of blackwater fever patients more susceptible to lysis Other theories implicate an upset of the normal balance between lytic tissue factors and their inhibitors the production of an autolysin as a result of plasmodial invasion of erythrocytes and changes in the plasma agglutinin pattern (Maegraith Findlay and Martin 1943 Weil 1907 Gear 1946 Butts 1945 Maegraith 1946)

The renal failure was thought to be due to mechanical blockage of the uriniferous tubules with precipitated haemoglobin products but recent work has shown this view to be untenable (Georgopoulos 1933 Foy *et al* 1943 Maegraith 1944) It is rather an example of the syndrome of renal anoxia the basis of the anuria being a redistribution of renal blood flow which results in relative cortical ischaemia with associated changes in the cortical portions of the renal tubules (Maegraith 1944 Maegraith Havard and Parsons 1945 Trueta *et al* 1946 *Lancet* leading article 1946) The characteristic lesions of the liver in blackwater fever are also probably essentially vascular in origin and are discussed in Chapter VI

Treatment

When malaria parasites are present in the peripheral blood a full course of mepacrine or paludrine should be given as for malaria Quinine should be avoided In the absence of parasites no antimalarial therapy is called for Parasites often appear in the early stages of

nitrogen is raised to some extent in all cases of blackwater fever whether complicated by obvious renal failure or not In cases recovering from anuria the urea nitrogen returns only slowly to normal The urine passed in oliguria may or may not contain blood pigments and albumin If the oliguria develops in the post-lytic phase the urine is clear and unconcentrated Its reaction in oliguria and during suppression which is not absolute (sometimes an ounce or less may be passed in 24 hours) is not as is frequently stated always acid but may be neutral or alkaline After recovery from anuria there may be polyuria associated with the passage of large quantities of dilute urine which slowly regains its normal concentration

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The death rate varies from 20 to 30 per cent Renal failure accounts for one-half of the deaths (Stephens 1937)

Diagnosis

Haemoglobinuria associated with progressive anaemia occurring in a subject who has been persistently exposed to malarial infection is

CHAPTER II

THE PARASITES OF HUMAN MALARIA

by

ROBERT H BLACK M D B S D T M & H

INTRODUCTION AND HISTORY ZOOLOGICAL CLASSIFICATION LIFE CYCLE In Man — In Mosq to — Trophozoite-i duced malaria — Persistence of malari l f ctio in Man — Infectio of M with malaria of other ve tebrates — Tr f of hu m l n to other rtebrates DESC IPTION OF PARASITES *P v x — P f l p r m — P ala a — P o le* — Ab ormal f m of p ras tes — Doubtful paras tes APPEARANCE OF PARASITES In th ck f lms — In t ues THE NUMB OF PARASITES IN PERIP ERAL BLOOD DIFFERENTIATION OF PA ASITES LABORATORY PROCEDU ES Thin films — Exfl gell ti n — Smears of tissue — Parasites in sectio s — W t film p epa ti ns — Thick film — En m ati n of par sites — Cultu at n of mal parasites PHYSIOLOGY OF THE MALARIA PARASITE MODE OF ACTION OF ANTIM LARIAL DRUGS

INTRODUCTION AND HISTORY

THE malaria parasites of man are true parasites living at the expense of their vertebrate host causing it harm and on occasions resulting in its death They are closely related to the malaria parasites of birds monkeys and other vertebrates which are also classified in the same genus—*Plasmodium*

Laveran (1880 a and b) was the first to describe the parasites found in the red blood cells of patients suffering from malaria The differentiation of the various species has been the result of the work of numerous observers since that date Manson (1894) expressed the opinion that mosquitoes would be found to transmit malaria That this was so in the case of bird malaria was reported by Ross (1898) who also followed part of the development of the parasites of human malaria in the mosquito (1897) Grassi and his colleagues (1898 1899 a and b) described the stages of development of the parasites of human malaria in the anopheline mosquito and Bastianelli and Bignami (1899) infected man with malaria by means of the bites of mosquitoes

With the work of Huff and Bloom (1935) Raffaele (1936 a and b) James and Tate (1937) and others interest was aroused in forms of malaria parasites seen in birds which developed outside the red cells These forms of the parasite had been described originally by MacCallum (1898) and Laveran (1900) Recently pre-erythrocytic forms of one of the human plasmodia (*P vivax*) were demonstrated in the

recovery and in these circumstances full antimalarial therapy is necessary

Cardiovascular failure potential or real must be treated on general lines Vomiting sweating and diarrhoea may tend to dehydrate the patient Fluid should therefore be replaced A daily fluid intake output account must be kept and the input balanced carefully against the loss since the patient may easily become waterlogged as a result of pushing fluids particularly in anuric cases (Paramore 1945 Macgraith 1945) Fluid may be given orally or intravenously in the form of glucose saline

If the erythrocyte count is 1 5 million cells per cu mm or lower transfusion of blood is advisable either in the form of a concentrated suspension of cells or as an infusion of citrated blood The transfusion should be given slowly and the amount given should be included in the total fluid intake for the day The greatest care is needed in transfusion Cross matching of donor s cells and patient s serum is essential

Alkalis are often recommended in big doses with the object of producing an alkaline urine Since the introduction of heavy alkaline treatment the death rate of the disease has risen In many cases no amount of alkali will alter the acid reaction of the urine In others the urine is alkaline or neutral to begin with There is evidence to show that large doses of alkali *per se* may be harmful to kidney function in normal subjects These facts indicate that alkali if administered at all must be given in moderation i e not more than about 20 gm in the course of 24 hours given as sodium bicarbonate If bicarbonate solutions (150 grains to the pint) are to be given intravenously they should never be sterilized by heat

Once the patient has become anuric there is little active treatment to be given beyond controlling his fluid intake Since however the renal failure of blackwater fever is fundamentally of the potentially reversible renal anoxic type it is possible that Kolff s (1946) artificial kidney might prove of some use

where the plasmodia reproduce by a process of division known as sporogony.

Man is infected by the entry of sporozoites in the saliva of an infective anopheline mosquito at the time of biting and after an incubation period suffers from an attack of malaria. During this attack sexual forms of the parasite are developed which are able to infect anopheline mosquitoes should they feed on the patient. After a period has elapsed these mosquitoes then become infectious for man.

The life cycle in man

Sporozoites from the salivary glands of infective mosquitoes enter through the skin of man at the time of biting. These sporozoites rapidly enter the circulation and have been demonstrated in the peripheral blood within a few minutes of biting by mosquitoes infected with *P. falciparum* or *P. vivax* (Fairley 1945). Half an hour after biting the parasites disappear from the blood. Prolonged microscopic search of the blood for parasites and subinoculation blood transfusions given to healthy recipients fail to show their presence in the peripheral blood for a period of 7 days for *P. falciparum* and 9 days for *P. vivax* (New Guinea strains of these parasites). In northern Europe, however, the period which intervenes between natural infection with vivax malaria and the first appearance of symptoms is often as long as 7 or 8 months (Hackett 1937).

The number of sporozoites required to produce malarial infection in man has not yet been determined with any degree of certainty. James (19[illegible]6) reported that 5[illegible] among 2[illegible]1 luetic patients who were bitten by mosquitoes of infective batches failed to develop malaria. Further, he said that the bite of one infective mosquito may be followed by a severe attack, whereas the attack following bites by 80 infective mosquitoes may be relatively mild. Fairley (1945) stated that a reasonable dosage of viable sporozoite will always infect the European who has not previously been exposed to malaria and that the natural immunity of the white man of European ancestry to jungle malaria is a myth—at least so far as New Guinea strains are concerned. De Sanctis Monaldi (1935) injected suspensions of *P. vivax* sporozoites of known concentration by various routes. He found that [illegible] 500 sporozoites given by intravenous injection produced an attack, as also did doses of 50 000 and 100 000. The incubation period was from 15 to 17 days. Intradermal injections of 5 000 sporozoites resulted in an attack of malaria after 74 days but a dose of 90 000 produced a negative result. Boyd (1940) found that the proportion of unsuccessful inocula-

liver by Shortt *et al* (*Brit med J* 1948 i 547) 7 days after infection by sporozoites

ZOOLOGICAL CLASSIFICATION

The classification of the Plasmodia is as follows (Wenyon 1926)

Phylum	Protozoa
Class	SPOROZOA
Sub-class	Coccidiomorpha
Order	COCCIDIIDA
Sub-order	Haemosporidiidea
Family	PLASMODIIDAE

The members of this family are included in the single genus *Plasmodium* These are parasites which produce pigment during their asexual cycle in the red blood corpuscles of vertebrates In human malaria the sporogonous cycle of the parasites is passed in the anopheline mosquito Four species of the genus *Plasmodium* are responsible for malarial infection in man These are *P vivax* *P falciparum* *P malariae* and *P ovale*

There have been various attempts to place *Plasmodium falciparum* in a separate genus *Laverania* because of the characteristic shape of its gametocytes but it is generally accepted now as *Plasmodium falciparum*

Christophers (1945) in a recent paper on the nomenclature of the malaria parasites stated that the three common plasmodia (*P vivax* *P falciparum* and *P malariae*) are according to the Rules of Nomenclature incorrect but the utmost confusion would result if attempts were made to introduce the correct names The only satisfactory procedure would be for the International Commission to suspend the rules in order to validate the names commonly in use

With the discovery of the exo-erythrocytic forms of the parasites of bird malaria the question arises of the correctness of the definition of the genus *Plasmodium* as containing organisms which pass their asexual cycle only in the red blood corpuscles of vertebrates

LIFE CYCLE

The malaria plasmodia of man have an asexual and a sexual cycle or existence The asexual cycle takes place in man in whom the parasites in the blood inhabit the red blood cells and undergo division by schizogony The sexual cycle occurs in mosquitoes of the genus *Anopheles*

sporozoites has already been mentioned The effects of certain drugs indicate that the parasites in this stage have a different susceptibility from that possessed by erythrocytic forms Fairley *et al* (1946 a) have reported that a single dose of 10 mgm of paludrine given 72 or 120 hours after biting with *P falciparum* prevented the development of an attack whereas all volunteers with overt falciparum malaria were not cured by 100 mgm of paludrine given daily for 7 days With *P vivax* James Nicol and Shute (1931) showed that plasmoquine produced a lengthening of the incubation period indicating an action on the pre-erythrocytic forms of this parasite Quinine and atebrin have no effect on this stage of the parasite and do not prevent the entry of parasites into the circulating blood at the end of the negative subinoculation period The relapses of vivax malaria despite clearing the blood of parasites by antimalarial drugs indicate the persistence of some form of the parasite resistant to these drugs periodically giving rise to erythrocytic forms which enter the circulating blood

At the end of the negative subinoculation period parasites are seen in the circulating red blood cells The youngest forms appear as small discs or rings which enlarge with the formation of pigment These asexual parasites or trophozoites divide by schizogony into a number of merozoites which are released when the red cells containing the parasites rupture The free merozoites enter fresh red cells where they grow and divide The time taken for one cycle of asexual development or the periodicity of the parasite varies with the species In *P vivax* and *P ovale* it is about 48 hours in *P malariae* 72 hours and from 36 to 48 hours in *P falciparum*

Some of the trophozoites do not undergo schizogony but develop into sexual forms known as male and female gametocytes These undergo no further development in man but are capable of infecting anopheline mosquitoes

The life cycle in the mosquito

When blood is drawn into the stomach (mid-gut) of an anopheline mosquito from a person in whom gametocytes are present in the circulating blood the gametocytes develop further Active movements of the gametocytes cause the rupture of the containing red cell and they then lie free in the stomach content Nuclear division occurs in the male gametocyte and a number of fine motile flagella are thrown out A piece of nuclear material enters into each one of these flagella Exflagellation as this process is called is followed by the separation of these actively motile filaments or microgametes from the parent

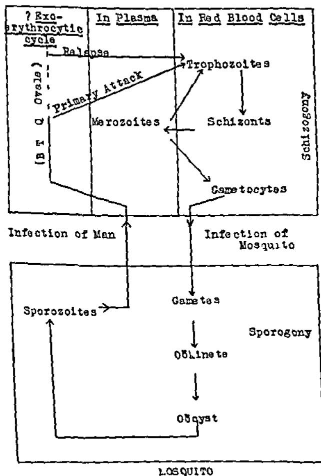
tions was high if the dose of sporozoites was small—as deduced by dissection of the salivary glands of mosquitoes—and that the incubation period tended to vary inversely with the dose of sporozoites Shute (1946) suggested that about 2 000 vivax sporozoites are necessary to ensure the normal incubation period He also reported that infection with a small number of sporozoites or with sporozoites that had been in the salivary glands for more than 2 or 3 weeks might result in a long incubation period

After the injection of sporozoites of *P gallinaceum* into chickens Huff and Coulston (1944) found that sporozoites were present in the heterophil leucocytes and that no sporozoite completed its development in this type of cell Thus these leucocytes if they did not subsequently disgorge the sporozoites acted as a defence mechanism against malarial infection Thus it is quite possible that there is a liminal dosage of sporozoites required to produce an infection

Although Schaudinn (1902) described the entry of a sporozoite directly into a red blood cell it is probable that the early stages of development of the malaria parasite in man take place outside the circulating blood Missiroli (1933 and 1934) described chromatin division in the sporozoites of *P praecox* at the site of injection into canaries but the sporozoites soon disappeared from this area Huff and Bloom (1935) described one of the malaria parasites of birds (*Plasmodium elongatum*) which developed in all the blood cell types Pigment was formed only in cells of the erythrocyte series which contained haemoglobin Raffaele (1936 a and b) found parasites in the reticulo-endothelial cells of birds infected with *P elongatum* and *P relictum* James and Tate (1937) recognized the significance of the same phases seen in *P gallinaceum* infections of fowls These exo-erythrocytic parasites—designated as such by James and Tate (1938)—persisted in the reticulo-endothelial cells and in the endothelial cells of the brain capillaries despite the clearing of the blood of circulating parasites by quinine and were responsible for killing the fowl by occlusion of the cerebral capillaries Huff and Coulston (1944) described the penetration of cells of the reticulo-endothelial system by sporozoites of *P gallinaceum* within half an hour of their injection into the host Here they developed into exo-erythrocytic forms which later gave rise to erythrocytic parasites

Thus the exo-erythrocytic cycle or tissue phase has been established in bird malaria Before the demonstration of the pre-erythrocytic forms of *P vivax* there was much indirect evidence for their existence The negative subinoculation period after infection with

tion of blood transfusion It may occur as a result of inefficient sterilization of apparatus used for injections as in the giving of neoarsphenamine (Wenyon 19 6 Black 1940) and amongst drug addicts (Biggam 19 9 Geiger 1932 Chung *et al* 1938) Malaria in newborn infants



CHA T 4—Diagramm tic represent tion of the lif cy le of the human malaria pla modi The e phase of P f parum end with the appeara ce of erythrocytic form s

cell The microgametes move amongst the red cells of the stomach content The female gametocytes extrude a small amount of chromatin and become macrogametes and are then ready for fertilization Fertilization (syngamy) takes place by the entry of a microgamete into a macrogamete and subsequent nuclear fusion to form a zygote The zygote elongates and becomes motile when it is known as an ookinete or travelling vermicule The ookinete penetrates and passes through the stomach epithelium and becomes embedded in the stomach wall forming an oocyst Within the oocyst very numerous thin fusiform sporozoites are formed which are about 15 μ in length. They are liberated when the oocyst bursts into the blood fluid of the haemocoele of the mosquito and eventually are distributed throughout its body They are however especially found in the salivary glands and their ducts Missiroli (1937) reported that the sporozoites from mature oocysts of *P praecox* in *Culex pipiens* were infectious for canaries so that there is no period of maturation in the salivary glands These findings were not in agreement with those of Sandicchi (1938)

The period which elapses between the ingestion of the infected blood by the mosquito and the appearance of sporozoites in the salivary glands is usually 10 to 14 days It is longer with *P malariae*, being from 28 days (Mer 1933) to 35 days (Boyd 1934) The length of time is influenced by the temperature of the atmosphere and by the species of the mosquito At the end of this sexual cycle the mosquito is infective for man Sporozoites are injected in the saliva of the mosquito through the bite in the skin and the asexual cycle in man begins

Trophozoite-induced malaria

Malaria may be transferred from man to man by means of inoculation of blood containing asexual parasites This transfer may take place accidentally or deliberately

Parasites may remain viable for at least 8 days when citrated blood is kept in a refrigerator (Sharnoff *et al* 1945) Johns (1931) added glucose to defibrinated blood containing trophozoites of *P vivax* and secured infections after it had been kept at 0° C for 18 days In the recipient of malarial blood the parasites continue to develop and undergo schizogony The infection differs from that of sporozoite-induced malaria in that erythrocytic parasites are present from the beginning If a sufficient number of parasites are injected intravenously they may be seen in the blood of the recipient a few minutes after the transfusion

Accidental transfer of malaria is occasionally reported as a complica-

infection appears to die out Ovale malaria may relapse for several years and quartan is the most persistent Accidental transfusion malaria has occurred using blood from donors 30 years or longer after they had been infected with *P malariae* (McClure and Lam 1945 Fischer and York 1946) The great preponderance of the quartan parasite as the cause of this complication of blood transfusion leads to the question of the persistence of trophozoites in the blood stream in infections with *P malariae* The donors in these cases are healthy despite the presence of parasites in the circulating blood at sub-microscopic densities It is unlikely that blood has been taken from these donors when the parasites were increasing to cause a relapse Thus it seems probable that after the initial attack and relapses with *P malariae* infections a state almost of commensalism rather than true parasitism ensues with parasites in small numbers in the peripheral blood The writer has seen such a case in a patient infected with *P vivax* of New Guinea origin with parasites present in the circulating blood for long periods at the borderline of microscopic densities and with no clinical concomitants

Trophozoite induced malaria when adequately treated does not show this tendency to relapse as all the parasites have been destroyed by the antimalarial drug used In *P gallinaceum* infections exo-erythrocytic forms occur in birds inoculated with trophozoites or sporozoites (James and Tate 1938) although they are seen at a comparatively later period with the former method of infection (James 1939) If secondary exo-erythrocytic forms are developed in trophozoite-induced malaria in man they must either be as susceptible to antimalarial drugs (such as atebrin) as are trophozoites—unlike the primary exo-erythrocytic forms—or incapable of producing parasites which will invade erythrocytes and cause relapses Oberle (1935) described non pigmented extra-cellular forms in the bone marrow in trophozoite-induced vivax malaria but these findings have not been confirmed

Infection of man with malaria of other vertebrates

Man has been experimentally infected with three parasites whose natural hosts are monkeys These infections were obtained by means of blood inoculation and the infections reported were with *P inui* (Das Gupta 1938 Ciuca *et al* cited by Hackett 1937) *P knowlesi* (Knowles and Das Gupta 193_ Jolly *et al* 1937 and others) and *P rodhaini* (Rodhain 1940) Infections with *P knowlesi* were transient and although some showed increases in the number of parasites spontaneous recovery occurred Jolly *et al* reported that passage of this

has been reported on several occasions (Heiser 1913 Clark 1915 Jones and Brown 1924 Tanner and Hewlitt 1935 Gammie 1944) and appears to be due to accidental admixture of maternal blood with that of the foetus Malarial infection of the placenta is very common in malarial areas (Blacklock and Gordon 1925) and a placental accident at the time of birth may account for the infection of the infant via the umbilical cord Deliberate trophozoite induction of malaria has been used in the treatment of syphilitic disease of the nervous system and experimentally in research on antimalarial drugs and the life history of the parasite

The persistence of malarial infection in man

Malaria parasites disappear from the circulating blood after adequate doses of antimalarial drugs but trophozoites of *P vivax* *P ovale* and *P malariae* are prone to reappear and cause relapses This occurs in the absence of any fresh infection of the host Relapses do not tend to occur with *P falciparum* infections after adequate treatment (as for example a course of atebrin)

A persistence of the tissue or exo-erythrocytic phase of the parasite with periodic invasion of the blood stream by erythrocytic parasites would account for these relapses Shute (1936) on the other hand considers it unlikely that exo-erythrocytic forms of the parasite—comparable with those seen in *P gallinaceum* infections in fowls—are responsible for relapses in vivax malaria He suggests that sporozoites may establish themselves in reticulo-endothelial cells These sporozoites when released on the death of the host cells may develop and bring about infection of the red cells causing a relapse So far no drug has been found which will prevent with certainty relapses of benign tertian ovale or quartan malaria despite the comparative ease with which the circulating blood can be sterilized In falciparum malaria the ending of the tissue phase with the first appearance of erythrocytic parasites apparently leaves no reservoir from which parasites can arise to cause relapses

Relapses are to be differentiated from recrudescences Inadequate treatment may cause the number of circulating parasites to fall below microscopic densities (one parasite per one to three cu mm of blood) but will not completely sterilize the circulating blood When the drug concentration falls below an effective level the parasites increase in number and give rise to a recrudescence of the infection

Relapses of vivax malaria may occur for several years the frequency to some extent depending upon the infecting strain Eventually the

stage preparation This active movement was responsible for the naming of the parasite *vivax* At this stage the ring has a diameter of about one third of that of the red cell in which it lies After about 6 to 8 hours growth a few granules of brownish pigment appear in the cytoplasm of the parasite which is now larger and irregular in shape (amoeboid form) or still maintains a ring form At this stage changes may be observed in the containing red blood cell its diameter is increased it has become somewhat pale and when well stained with a Romanowsky stain shows a number of fine red granules known as Schuffner s dots

At the end of 24 hours growth the parasite fills about one-half of the red cell It is irregular in shape with active pseudopodia extending out into the red cell and contains numerous granules of yellowish-brown pigment scattered throughout the cytoplasm

Increase in size continues until at about the end of 36 to 40 hours the parasite almost completely fills the enlarged red cell It has now lost its amoeboid activity and the cytoplasm becomes more compact although its border is somewhat irregular

The nucleus increases in size with the appearance of several granules and then divides to form daughter nuclei which divide further until typically 16 nuclei are present although the number varies between 12 and 24 The cytoplasm condenses around these nuclei and at about the end of 48 hours a mature schizont is formed containing 12 to 24 merozoites which are round or oval in shape and usually arranged in two rows During nuclear division the pigment becomes accumulated in one or more areas and there is a gradual increase in the size of the parasite so that it reaches 10 to 11 μ in diameter The remaining portion of the red cell still shows Schuffner s dots which are more numerous and coarser than in the younger trophozoite When segmentation is complete the red cell ruptures and the merozoites and pigment are released into the blood plasma The merozoites quickly become attached to new red cells and after entering them repeat the process of schizogony

Most parasites mature at the same time—at the end of 48 hours—but some are fully divided before this time and some take longer In some cases two batches of parasites may be seen which attain maturity at different times which may be 24 hours apart producing a schizogony each day In the early days of the primary attack the presence of parasites developing out of phase is very common (James 1926 Lowe 1934) In relapses of vivax malaria in the writer s experience the parasites appear to be more in phase

parasite through man reduced its pathogenicity for monkeys The infection with *P rodhaini* is open to question because of the similarity of this parasite to *P malariae* Ciuca *et al* (1937) have passed *P knowlesi* from man to man many times by trophozoite inoculation

Transfer of human malaria to other vertebrates

P vivax and *P falciparum* have been transmitted to monkeys by means of blood inoculation of trophozoites In the howler monkey (*Alouatta* Sp) *P falciparum* produces a transient infection with increase in the number of parasites It shows a 48-hour cycle and the parasites cannot be distinguished morphologically from those seen in man (Taliaferro and Taliaferro 1934) Mesnil and Roubaud (1917) inoculated blood containing *P vivax parasites into a chimpanzee where the trophozoites* underwent schizogony The animals showed no marked fever and the parasites disappeared after 22 days Rodhain and Muylle (1939) reported the transfer of trophozoites of *P vivax* to two chimpanzees In the first parasites were seen for 9 days In the second the parasites persisted for 46 days as shown by subinoculation to two men who developed typical vivax infections

There has been no reported transfer from either host to the other through mosquitoes

DESCRIPTION OF PARASITES

Four species of the genus *Plasmodium* are recognized as being responsible for malarial infection in man These are *P vivax P falciparum P malariae* and *P ovale* There are in addition other doubtful species and varieties which will be described later The descriptions given here are limited to the appearance of *the parasites as seen in the human* host and apply to both sporozoite- and trophozoite-induced malaria

Plasmodium vivax

This parasite causes benign tertian malaria in man It was first observed as a distinct species by Golgi *in 1886* (a and b) *Grassi* and Feletti named it *Haemamoeba vivax* in 1890

P vivax takes 48 hours to complete its asexual cycle and divide by schizogony *The earliest forms seen* in the blood are in the shape of a disc with a single nucleus within the red blood cell Within a few hours a vacuole appears and the parasite resembles a signet ring as the nucleus is pushed to one side The parasite is now actively amoeboid and this movement may be observed microscopically with a warm

stage preparation This active movement was responsible for the naming of the parasite *vivax* At this stage the ring has a diameter of about one-third of that of the red cell in which it lies After about 6 to 8 hours growth a few granules of brownish pigment appear in the cytoplasm of the parasite which is now larger and irregular in shape (amoeboid form) or still maintains a ring form At this stage changes may be observed in the containing red blood cell its diameter is increased it has become somewhat pale and when well stained with a Romanowsky stain shows a number of fine red granules known as Schuffner s dots

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When schizogony has occurred several times a number of trophozoites *do not develop into schizonts but form male and female gametocytes* Thus gametocytes are not seen in the circulating blood in the first days of an attack of vivax malaria Immature gametocytes are not often seen in the peripheral blood circulation as their maturation appears to take place in the blood vessels of the spleen and bone marrow Their growth is slower than that of a trophozoite No vacuole is produced and there is no active amoeboid movement although some movement is seen in a warm stage preparation The containing red cells show the changes described with the developing trophozoite increase in size pallor and Schuffner s dots Maturation of gametocytes takes about 96 hours for completion

The cytoplasm of the gametocytes is rounded and occupies almost the entire red cell When stained with a Romanowsky stain the *cytoplasm of the female gametocyte is darker in colour than that of the male* The pigment is yellow-brown in both and is scattered throughout the cytoplasm in the form of granules of which there are more than in the mature schizont The nucleus of the male gametocyte is larger than that of the female In both forms it is always single If viewed in a wet film preparation the male gametocytes after escaping from the red cells are seen to have active motile pigment granules and undergo the process of ex-flagellation forming 2 to 6 microgametes

Red blood cells are occasionally seen containing multiple infections with vivax parasites There may be two or more trophozoites or more rarely a trophozoite and a gametocyte

Characteristic features of *P vivax* which help in its differentiation from the other plasmodia are active motility enlargement of the red cell with the appearance of Schuffner s dots the occurrence of all stages of schizogony in the peripheral blood the number of merozoites formed and the appearance of the gametocytes

In thin film preparations stained with Leishman s stain the cytoplasm of the parasites is blue the chromatin crimson and the red cells salmon-pink in colour The monocytes may contain pigment granules

In a well-developed infection with *P vivax* parasites are usually moderately numerous and one may be seen in every few microscopic fields The parasite may be present in all stages of growth or only a few stages may be seen depending upon the synchronicity of development It is not uncommon to see two parasites in one red cell A varying number of gametocytes may be present The ring forms are relatively large with a single mass of chromatin Amoeboid forms

are larger with pigment granules of a yellow-brown colour scattered through the cytoplasm The red cell is enlarged and becomes pale as the parasite increases in size Schuffner s dots staining a dark red are seen in the red cell Prior to division of the chromatin the trophozoite may present some resemblance to a gametocyte If the parasites are developing in phase examination of the blood during a paroxysm will reveal mature schizonts and some young rings About 12 hours later large rings will be seen with some amoeboid forms Twenty-four hours after the paroxysm the parasites are the larger and irregular amoeboid forms Thirty-six hours after the paroxysm nuclear division of the schizont will be seen to have commenced and this is completed shortly before the next paroxysm The table shows the stage of development of *P [illegible]ax* at various times during one schizogonous cycle

TABLE I

10 11 12/4	PARASITES					
Time	*Total per cu mm*	*R*	*A*	*Ps*	*S*	*Gametocytes per cu mm*
10						
0800	860	++			++	
1400	3900	++			+	
1800	4050	++	+			
2400	3340	+	++			60
11						
0800	3460	+	++	+		1[illegible]0
1400	3060		++	+		140
[illegible]000	4780		+	++		60
2400	4440			++	++	60
12						
0200				+	++	
0400	2700	+			++	40
0600	4560	++			+	90
0800	17 000	++			+	[illegible]0

TABLE 1—Form of p[illegible]as[illegible]es present [illegible] the blood [illegible] p[illegible]tient with [illegible] ax m[illegible] [illegible] t variou[illegible] times d[illegible]ring o[illegible] schizogo[illegible]o[illegible] cycle (N[illegible]t[illegible] R rings A amoebo[illegible]d form Ps form seen imm[illegible]d[illegible]t[illegible]ly bef[illegible]e [illegible] [illegible] [illegible] S [illegible] [illegible]) (Orig[illegible]n[illegible]l)

Plasmodium falciparum

P falciparum is the parasite which causes malignant tertian subtertian or aestivo-autumnal malaria in man It derives its name from the shape of the gametocytes

Laveran in 1881 and Golgi in 1886 (a) gave early descriptions of this parasite They were followed by fuller ones by Canalis (1889) and Celli and Marchiafava in the same year Welch in 1897 proposed the name *Haematozoon falciparum* There have been attempts to place *P falciparum* in a separate genus *Laverania* because of the characteristic shape of the gametocytes but the generally accepted classification is now *P falciparum*

The time taken for this parasite to complete a schizogonous cycle is about 48 hours or somewhat less The behaviour of the parasite differs from that of *P vivax* in that the process of schizogony does not take place in the peripheral circulation and only the younger trophozoites are usually found in blood films As the trophozoite increases in size the red cell containing it becomes sticky and it is held up in the capillaries of the internal organs This stickiness is seen when the parasite is cultivated *in vitro* where clumps of infected red cells may be seen especially around cells of the monocyte variety After schizogony the merozoites enter fresh red cells and appear in the peripheral blood as young trophozoites

The youngest trophozoites are thin minute ring forms whose diameter is about one-sixth of that of the red cells containing them Often the cytoplasm is applied along a part of the circumference of the red cell with loss of the ring form (applique forms) Many other forms may be seen with irregularity in the shape of the cytoplasm Two pieces of chromatin are often present in a single parasite The parasite increases in size and takes on amoeboid activity A small amount of pigment may be formed by the parasite before it withdraws from the peripheral circulation In heavy infections some of the more mature parasites may be seen in the circulating blood

As the parasite increases in size pigment is formed which is much darker in colour than that of *P vivax* being dark brown or black The cytoplasm increases in amount and remains fairly compact and rounded There is no increase in the size of the containing red cell which may show Maurer s dots These are seen when a film is heavily stained and are less numerous and larger than Schuffner s dots They are rod- or wedge-shaped and give the appearance of clefts in the red cell The red cell in a stained film may have a crenated edge

When the parasite is nearing its full size the pigment granules become compacted together to form a prominent dark granular mass The nucleus divides to form a varying number of segments the number ranging from 8 to 36 The cytoplasm condenses around the divided nuclear material to form merozoites The mature schizont occupies about two-thirds of the diameter of the red cell which releases the merozoites and pigment when it ruptures

Gametocytes are formed from certain of the merozoites Early forms as with *P vivax* are not often seen in the peripheral circulation The gametocytes male and female are crescentic in shape with rounded or pointed ends and the remaining part of the red cell may be seen arched across part of the concave border The male gametocyte has a hyaline cytoplasm with a relatively large lightly-staining nucleus and pigment scattered throughout the cytoplasm In the female the nucleus is smaller and stains more darkly the cytoplasm is denser and the pigment tends to be accumulated about the nucleus in the centre of the gametocyte More female than male gametocytes are usually seen in blood films the ratio being of the order of 3 1 (Stephens and Gordon 1924 Pawan 1927) The shape of the gametocytes varies between a pointed crescent and a plump bean-shaped body and these various shapes may be seen in one film

With *P falciparum* multiple infections of red cells by trophozoites are common The number of parasites both trophozoites and gametocytes seen in films is often much higher than with *P vivax*

In thin blood films made from *P falciparum* infections there are usually relatively large numbers of parasites There are often several parasites to be seen in one microscopic field Usually only ring forms and gametocytes are present but if the infection is severe the more mature parasites may be seen It is not uncommon to see infections in patients with microcytic anaemia in which cases the invaded red cells may be smaller than normal

Plasmodium malariae

This is the parasite which causes quartan malaria It was first studied by Laveran in 1880 (a and b) and called by him *Oscillaria malariae* It was described by Grassi and Feletti in 1890

The parasite grows slowly and takes 72 hours to complete the schizogonous cycle which occurs in the peripheral blood The ring forms are difficult to distinguish from those of *P vivax* It shows little amoeboid activity during its growth and is thus more compact than

P vivax The pigment granules are coarse and dark in colour resembling those of *P falciparum*

A characteristic disposition of the parasite in the red cell is in the form of a band stretched across the red cell (band form equatorial form) The red cell is not increased in size indeed it may appear smaller than normal It shows no Schuffner s or Maurer s dots

A nearly-mature schizont of *P malariae* almost fills the red cell and is circular in outline The pigment accumulates in one area and the nucleus divides into 6 to 12 segments The cytoplasm divides around the nuclear material and the merozoites may become arranged around the pigment mass to form a rosette if the pigment happens to be centrally situated

The gametocytes are round and almost fill the containing red cells The cytoplasm of the female is more dense than that of the male and its nucleus more compact The pigment is scattered throughout the cytoplasm Young gametocytes are not often seen in the peripheral circulation and are difficult to distinguish from schizonts in their early stages

The characteristic features of *P malariae* are the slowness of its growth an infected red cell of normal size with no Schuffner s or Maurer s dots the band forms which occur very rarely with other plasmodia the relatively small number of merozoites and the almost complete filling of the normal-sized red cell when the parasite is mature

P malariae is usually present in the blood in relatively small numbers and a prolonged search of a thin film may reveal only a few parasites

Plasmodium ovale

This parasite was described by Craig in 1900 but was not named (Craig 1933) It was described and named by Stephens (1922) James Nicol and Shute (1933) described the parasite and its passage from man to man through mosquitoes (1932)

P ovale produces an infection resembling that caused by *P vivax* with a fever which is tertian in type The schizogonous cycle takes 48 hours to complete and occurs in the circulating blood

Young rings measuring about 2 to 2 5 μ are contained in red cells which are usually heavily studded with Schuffner s dots The chromatin material is irregular in shape and is relatively large in amount With increase in size the containing red cell becomes enlarged and pale and frequently has a ragged outline The pigment formed is in finer granules and a lighter colour than in *P malariae* The parasite shows little amoeboid movement and is round or oval in shape

In the stage of segmentation of the nucleus 25 per cent of the infected red cells are oval in shape and this characteristic feature led to the naming of the parasite The dividing schizont is usually round in shape measuring about 6 2 μ in diameter Frequently the red cell has a ragged outline or fringe which is decolourized and may contain Schuffner s dots which appear to be outside the corpuscle The schizont divides into 6 to 12 merozoites the pigment being accumulated in a central mass

The gametocytes resemble those of *P vivax* especially as they are seldom found within oval corpuscles

P ovale though it shows the characteristic change in the shape of the containing red cell requires careful study and comparison with the other plasmodia for its differentiation In thin blood films the parasites may be relatively scanty necessitating prolonged examination for correct species diagnosis

Abnormal forms of parasites

The descriptions given are for typical forms of the parasites Changes may be seen with different methods of staining and the administration of drugs causes variations in the appearance of the parasites seen in the circulation Wenyon (1926) described degenerate forms of *P vivax* caused by the administration of quinine with smaller schizonts containing fewer nuclei than normally seen and changes in the staining properties of the parasites James (1934 a) described the degenerative changes produced by atebrin on *P vivax* and *P malariae* and Huhne the changes in *P falciparum* Fairley *et al* (1946 a) reported the changes seen with paludrine acting on *P vivax* where development of the trophozoite ceased at the stage immediately preceding nuclear division (preschizont) Black (1946) described analogous changes produced by paludrine on *P falciparum*

It is remarkable that while the individual species may cause marked differences in clinical and immunological features in various parts of the world giving rise to the naming of strains of parasites the morphology of the plasmodia remains very much the same

Doubtful parasites

From time to time different varieties and species of plasmodia have been described *Plasmodium ovale* was classified as a doubtful species until it was studied fully and its characteristics were shown to be retained on passage from man to man and through mosquitoes

Plasmodium tenue was reported by Stephens in 1914 as seen in a single blood film taken from a child and sent to him from India Sinton (192-) described a case in India where similar parasites were seen regularly The characteristic features described by Stephens were marked amoeboid activity as judged by the morphology of the young trophozoites which were the only forms present in the slide examined scantiness of the cytoplasm and the presence of a relatively large amount of nuclear chromatin

Plasmodium perniciosum was described by Ziemann in 1915 as the parasite causing malignant tertian malaria in West Africa He stated that it produced less pigment than *P falciparum* as seen in Italy the schizonts were smaller and contained 12 to 16 merozoites

Plasmodium falciparum quotidianum was studied by Craig in 1909 and regarded by him as being a sub-species He described it as smaller in size at all stages than *P falciparum* and relatively rich in chromatin in the ring stage The number of merozoites was on an average from 12 to 14 in number and the time for a schizogonous cycle was 24 hours The gametocytes were relatively smaller than those of *P falciparum*

Plasmodium vivax var *minuta* was described by Emin in 1914 He stated that the containing red cell was not enlarged Schuffner s dots were present the parasite was actively motile and the number of merozoites from 4 to 10

Plasmodium falciparum var *aethiopicum* was described by Raffaele and Lega in 1037 who stated that the ring forms were larger and richer in chromatin than *P falciparum*

Plasmodium wilsoni was described by Roberts (1940) in East Africa as a quartan type of parasite in an enlarged red cell forming dark pigment and having from 10 to 12 merozoites in a mature schizont It caused heavy infection and multiple infection of red cells was common

Other un-named varieties have been described but none of these parasites have been fully studied or accepted by the majority of malariologists nor has any specific strain been isolated as the causal parasite of blackwater fever

APPEARANCE OF PARASITES IN THICK BLOOD FILMS

In thick blood films from which the haemoglobin has been removed the outlines of most of the red cells are obliterated Those parts of the film in which the nuclei of the leucocytes are stained purple show parasites at their optimal colour differentiation The cytoplasm of the

parasite is stained blue and the chromatin material crimson The diagnostic features will be outlined

The ring forms of *P vivax* are relatively larger than those of *P falciparum* The amoeboid forms may appear to have their cytoplasm fragmented into several parts At the edge of the film parasites may be seen within the ghost outline of red cells containing Schuffner s dots The schizonts are larger than the polymorphs

In *P falciparum* infections the ring forms are small and fine two chromatin dots are common and often the cytoplasm is comma-shaped or disposed like the wings of a bird on either side of a chromatin body Growing trophozoites and mature schizonts are usually not seen The crescentic shape of the gametocytes is characteristic

In infections with *P malariae* and *P ovale* there are no amoeboid forms seen and the growing trophozoites are more compact and have darkish pigment The number of merozoites is relatively small The gametocytes are circular in outline

APPEARANCE OF PARASITES IN TISSUES

Tissues removed at biopsy or autopsy from patients during an attack of malaria will show parasites in the blood vessels corresponding to the stages seen in the peripheral blood In addition in falciparum infections dividing and mature schizonts will be seen within red blood cells which may be adherent to capillary walls In the placenta in malaria-endemic countries infection with *P falciparum* may be found when no parasites have been seen in the peripheral blood (Clark 1915 Blacklock and Gordon 1925)

Exudates containing red blood cells may also show the presence of malaria parasites e g those seen in dysenteric infections during an attack of malaria

Attempts have been made to demonstrate the presence of malaria parasites during latent periods between relapses Gasic (1938) examined the sternal marrow of twelve such cases and concluded that the procedure was of no practical diagnostic value As has been mentioned elsewhere exo-erythrocytic forms of human malaria parasites have only very recently been demonstrated

THE NUMBER OF PARASITES IN PERIPHERAL BLOOD

In a primary attack of sporozoite induced malaria the number of parasites found in the peripheral blood at the time of onset of symptoms

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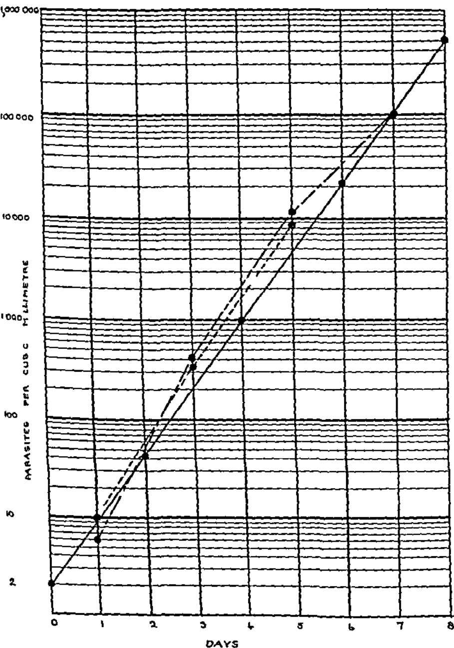
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C RT 5—Theo t c l re t h number of pa te n the l ood a P f l p rim nf t on wh r th schiz t e ach f ng 2 m o t Th obse d cr ase seen in two sc lso plotted (Co tin ous lin s theor ti al curve)

may be very small Indeed diligent search of a cubic millimetre or more of blood in a thick film may fail to reveal a single parasite On the other hand the parasites may be relatively numerous Ross and Thomson (1910) described a pyrogenic level which varied from 200 to 500 parasites for *P vivax* infections and 600 to 1 500 for *P falciparum* infections These figures were derived from a study of malaria in seamen at Liverpool Since that date many observations have shown that fever may on occasions precede the appearance of parasites at microscopic densities It may be taken as a general rule however in primary infections that the onset of symptoms coincides roughly with the time that parasites are first seen in thick films

v Assendelft (1934) found that the number of parasites present in primary vivax malaria at the onset of fever varied between 0 3 and 900 per cu mm

Relapses of vivax malaria in the writer s experience with New Guinea strains are usually associated with the presence of fairly numerous parasites in the peripheral blood when the patient becomes ill

In theory if all the merozoites invade fresh red cells and come to maturity the number of parasites would increase during each cycle at a rate of the order of twenty-fold in falciparum and vivax and ten-fold in malariae infections Taliaferro (1925) counted the increase in number of parasites in bird malaria and found that 10 merozoites out of an average of 15 5 per mature schizont did not develop into mature schizonts Lowe (1934 b) describing the number of parasites seen in cases of untreated malaria counted the number of schizonts in vivax infections before a paroxysm and the number of young trophozoites found afterwards He reported that 62 5 per cent of merozoites were destroyed but was dealing with the later part of the infection where counts were on the average 11 200 per cu mm

If the theoretically computed increase of parasites in falciparum malaria is compared with one constructed from observations made two hourly in infections with New Guinea strains the two are seen to agree closely over a period of two or three cycles (Chart 5) Thus it appears that with these strains of falciparum malaria during the logarithmic phase most of the merozoites succeed in entering fresh red cells and developing into mature schizonts The rise in the number of parasites is so inexorable that when the count reaches 100 000 per cu mm the curve shows but little tendency to flatten out—this of course it must do (if the patient survives) for the number of red cells per cu mm is a limiting factor despite the multiple infection of red

a large wave of gametocytes does not appear unless preceded by a high number of trophozoites Thomson (1911 a) suggested that gametocytes were developed in falciparum infections as a result of the development of immunity towards the original parasites In infections with *P malariae* the number of gametocytes seen is relatively small When a gametocyte wave occurs in falciparum infections the crescents usually appear in the peripheral blood at microscopic densities about 8–10 days (Ross and Thomson 1910 Thomson 1911 a) after the day of the first appearance of asexual forms at similar densities The numbers increase fairly rapidly and after the peak of the curve is reached the number slowly fall if no gametocidal drug is given—they may be seen in the peripheral blood for 3 weeks (Dick and Bowles 1947) or more after an adequate course of trophozoiticidal treatment has been given

The number of gametocytes seen in vivax infections is small compared with some of the counts obtained with falciparum infections On the other hand Boyd *et al* (1935) stated that for the practical transmission of malaria by *A quadrimaculatus* the number of vivax gametocytes required as a minimum was only one-eleventh of that for *P falciparum*

Various estimates have been given for the number of gametocytes required to infect anopheline mosquitoes Darling (1915) stated that the limit of infectiousness was 12 gametocytes per cu mm for *A albimanus* Green (1929 a) found that the lowest density of gametocytes at which *A maculatus* became infected was for *P falciparum* 42 per cu mm for *P vivax* 10 per cu mm and for *P malariae* 27 per cu mm He failed however to infect some mosquitoes when the blood of the patients contained many more gametocytes James (1931) working with *A maculipennis* found that there was no infection with *P vivax* if there were fewer than one exflagellating male gametocyte per 1 000 leucocytes However with *P falciparum* and *P malariae* the finding of considerable numbers of exflagellating male gametocytes was not a sure sign that *maculipennis* would become infected Boyd *et al* (1935) stated that minimum densities per cu mm of one male and one female gametocyte of *P vivax* and 11 male and 11 female gametocytes of *P falciparum* were necessary for the practical infection of *A quadrimaculatus* Robertson (1945) found that the gametocyte density required regularly to infect *A gambiae* and *A melas* with *P falciparum* was 1 per 35 leucocytes

Thus the number of gametocytes is not the sole factor determining the infection of mosquitoes The species and varieties of anopheline

cells by parasites Fairley *et al* (1947) state that in their volunteers suffering from primary falciparum malaria the parasites had reached an average density of the order of 300 000 per cu mm by the seventeenth day after infection

In primary vivax infections however the curve plotting the rise in the number of *parasites tends to flatten out when a density of the order* of from 5 000 to 20 000 parasites per cu mm is reached There is obviously some limiting factor in operation and in this regard it is interesting to note that several observers (Eaton 1934 Kitchen 1938 Ferrebee *et al* 1946) have shown that *P vivax* has a predilection for reticulocytes The merozoite of *P falciparum* does not appear to exercise this selection of its host cell (see Chapter III)

P malariae has few merozoites a relatively slow growth and the number of parasites found in the peripheral blood is relatively small This parasite apparently prefers to infect mature red cells

If the number of parasites in the blood is followed at frequent intervals in a case of falciparum infection in which the parasites are developing in phase a fairly rapid drop to a low figure will be observed (such as from 8 500 to 56 per cu mm in 9½ hours) which is associated with the withdrawal of the developing trophozoites to undergo schizogony in the internal capillaries Similarly the next crop of young ring forms will be seen to emerge with a large increase in the number of parasites in the peripheral blood (as for example an increase from 60 to 59 200 in 14 hours) It is by plotting the crests of these waves that one can determine the actual increase in the number of parasites The troughs are important as a mistaken idea of the severity of the infection may be derived if notice is taken only of isolated parasite counts Usually it will be seen during the phase when the count has fallen that the parasites are relatively large rings and soon they too will disappear from the peripheral blood There may also be seen very fine rings of the next generation In cerebral malaria when there are very numerous dividing parasites in the cerebral capillaries the number of parasites seen in the peripheral blood may be very small or even nil This marked periodic fall in the number of parasites in the peripheral blood (during the phase of increase) is not seen in vivax infections as *here schizogony occurs in the peripheral blood*

The behaviour of parasites in primary trophozoite-induced malaria is essentially similar to that seen in sporozoite-induced infections

The number of gametocytes in the peripheral circulation does not appear to bear a direct relation to the number of trophozoites although

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mosquitoes show varying susceptibility to infection and there is individual susceptibility within species In 1935 Huff described his work on the natural susceptibility and immunity of culicine mosquitoes to avian malaria Immunity to infection was species specific and was an inherited condition as also was the susceptibility to infection Stratman-Thomas (1940) found that unfavourable temperatures could destroy *P vivax* in the cycle in the mosquito the early stages being most susceptible and unfavourably high temperatures more effective than unfavourably low temperatures Darling (1910) described the active phagocytosis of gametocytes in the mosquito s stomach by the polymorphs in the ingested blood So for the infection of a mosquito there must be adequate gametocytes in the blood meal the mosquito must be susceptible to infection the gametes must pass by the polymorphs in the mosquito s stomach and the temperature of the environment must be favourable James (1931) also expressed the belief that the quality of the gametocytes is important in the infection of mosquitoes

DIFFERENTIATION OF PARASITES

It is important not only to diagnose malaria by a demonstration of malaria parasites in the patient s blood but also from a clinical and epidemiological viewpoint to differentiate the species of *Plasmodium* present The table gives a summary of the chief features of the four *Plasmodia* of man which serve to give a correct species diagnosis

Thick film preparations (page 56) are usually adequate for the diagnosis of *P vivax* *P falciparum* and *P malariae* but there are several circumstances in which it is necessary to examine thin films and even follow the various stages of development through a complete schizogonous cycle In mixed infections an examination of a thin film is desirable so that changes in the red cells may be observed in addition to the morphology of the parasites Infections with *P malariae* may be misdiagnosed if the band forms are not recognized in the thick films or if ring forms only are present If a development cycle is followed its relative slowness will be observed and in a film made when the parasites are undergoing division the characteristics of the schizont will aid in its differentiation

P ovale requires very careful study for its diagnosis which should be made after observation of all the stages of development and comparison with films of the other species of parasites

TABLE II

DIFFERENTIATION OF HUMAN MALARIA PARASITES

	P x	*P f l p m*	*P l r*	*P le*
D at n f sch zog no cy le	48 ho rs	48 hours or les	72 hours	48 hour
St ges een n per pheral blood	Trophozo tes h ont a d g m t cyt s	U u lly onl ea ly tr pho oit and g tocytes	Troph oites schizont and g metocytes	T oph oit s h z nt and ga- n t cyt
Inf cted red c ll	En ged pal Schuffner d t	Not enla ged Maurer dots.	N t enla ged No S huffne s o Mau d t	Enl ged Pal ll tnes o l S huffne d t
Number f p a-sites n red ll	Doubl f ction t ncommon	Infection with or more pa tes c y comm on	Doubl nf non e y a	D ble i f t on a
Young tropho-zo te	Small nd l rg r ngs One chro-matin dot	Small f ne r g Often 2 hr m tn d ts Appl q e f m	S ll nd l g ng One h o-m tn dot N t w h n l fo n	Small d la g ng
P g nent	Y llow b own f ne pa t l	D rk b own or bl ck Co se	Dark b own o bl k Co	D k h b own N t o o rse
Am b id movem nt	A u e	P sent	Sl gh	Sl ght
Segment eschi-z nt	Ro nded w th r g lar ou l	O l o ound U u lly b nt f om l t g blo d	R nd l	Rou d
M ture h ont	12 24 m o o-t	8-36 m o t Pigment p m nent	6-1 m o t M y b osett w th tral p g m t	6-1 z tes C nt l p g t
S ze f n u e sch zont l t v to d cell	Almo t c n pl t ly f ll en l g d d c ll	Occup t w th d d n t f n l-s d ed ell	Alm t f ll o m l d d ll	A b o u t t h q t f d n t f enl g d d ll
G m etocyte	Ro nd Al n t f ll l g d d ll	Crescen M y b pl mp o b h p d	R d F ll l f d ll	R und R m bl P R d ll l g d lv l

Ta le 2—D ff entiati of hun an n l n p t

LABORATORY PROCEDURES

The techniques detailed in this section do not include all the methods which have been described for the demonstration and enumeration of

parasites but are the ones commonly in use and give satisfactory results

Parasites in the blood may be examined in thin and thick films and in wet preparations In tissues they may be demonstrated by means of stained smears or in sections The enumeration of parasites in the circulating blood is an essential procedure on many occasions

Thin films

Thin films are made as for ordinary blood examinations and may be stained by any of the Romanowsky blood stains Leishman s stain gives uniformly good results if a standard technique is used The film is fixed with a 0 15 per cent solution of the stain in methyl alcohol for 1 minute and then this is diluted with twice its volume of buffered distilled water * It is essential that the volumes of stain and buffer solution should be measured accurately and to ensure this two Pasteur pipettes may be graduated and one used for the stain and the other for adding and mixing the buffer solution The diluted stain is allowed to remain on the slide for 25 minutes after which the film is differentiated with water and allowed to dry

In a film which has been properly stained for malaria parasites the granules of the neutrophil leucocytes should be well stained

Exflagellation

Exflagellation of the male gametocytes in thin blood films may be demonstrated by Shute s method as described by James (1934 b) Four Petri dishes are prepared by fitting two filter papers into the top and bottom dish of each pair The filter papers are moistened and a triangular piece of glass tubing is placed in each Petri dish The dishes are then put in a moist atmosphere incubator for 2 hours at 25° C While the patient is being bled from a finger prick the dishes are kept warm Thin films are made lightly breathed upon and quickly placed on the triangle in the Petri dishes and the lids replaced Four preparations are made and placed in the incubator for 15 20 25 and 30 minutes At the end of these intervals the films are removed from the Petri dishes and examined to see if they are still moist They are then dried in the air and stained with Leishman s stain In the tropics the

Buffer solution

Sod um pho ph te ($N\ HPO_4\ 12H\ O$)	2-0 g ammes
Pot sium d hyd ogen phosphate ($KH\ PO$)	1 0 gramme
Thymol	1 0
D till d water	to 1 000 ml

atmospheric temperature is often sufficiently high to secure good exflagellation

The flagella (microgametes) appear as long filamentous bodies staining red or violet and are attached to or separated from the periphery of the male gametocyte

Smears of Tissues

Smears are made by dabbing the cut surface of an organ such as spleen or brain on a slide and then staining with Leishman's stain as for a thin blood film Material obtained from sternal puncture is spread and stained in the same way as tissue smears Dilute Giemsa's stain may be used for smears of tissue After fixing in absolute methyl alcohol for 30 seconds the slide is washed and then covered with Giemsa's stain diluted 1 in 10 in a buffer solution of pH 6 8 to 7 0 After 30–45 minutes the slide is washed and dried

Parasites in sections

Giemsa's stain gives differentiation of the parasites from the host's cells The method described is that suggested by Hewitt (1940)

The tissue is fixed in Zenker's fluid containing 5 per cent formol for 18 to 24 hours after which it is washed and the bichloride of mercury removed with iodinized alcohol It is then dehydrated in alcohols and embedded in paraffin Sections are cut from 5 to 10 μ in thickness and passed through xylol absolute alcohol 95 per cent alcohol and 70 per cent alcohol into distilled water A 2 5 per cent solution of potassium bichromate is used as a mordant and the sections are placed in this for from ½ to 1 hour They are then washed quickly in distilled water and placed in the following stain

Distilled water	1.0 c c
0 5% Na_2CO_3	2–4 drops
Methyl alcohol (c p)	3 c c
Giemsa's stain*	2 5 c c

The sections are left in the stain for 24 hours When removed the excess stain is washed off in distilled water coloured lemon yellow with ~ 5 per cent potassium bichromate

G em a t

Az II-eosin	3 0 g ammes
A u e II	0·8 g mme
Glycerin (p)	200·0 grammes
Absolut n thyl al h l (c p)	250·0

Differentiation with 70 per cent alcohol is the critical step in the procedure and the time varies with the thickness of the section and the type of tissue involved The time required is from ¼ to 2 minutes and the tissue is then washed quickly in distilled water as soon as the stain is being removed in noticeable quantities It is then dehydrated and mounted Dehydration is accomplished by passing through a series of xylol-acetone mixtures as alcohol removes the stain The following mixtures are used after the distilled water wash

5% xylol	95% acetone	1 minute
30%	70%	2 minutes
70%	30%	2
xylol		5

Fresh mixtures of 5 per cent xylol and 95 per cent acetone must be used as continued use is apt to remove the stain The section is then mounted in neutral balsam

Wet film preparations

A drop of blood is placed on a slide covered with a coverslip and the edges are sealed with paraffin which fixes the coverslip and prevents evaporation If the slide is kept warm the amoeboid movements of the parasites may be seen and also the continuation of their development for a few hours

In the days before effective staining techniques were introduced microscopic examination of fresh blood was the laboratory method for the diagnosis of malaria The larger parasites were seen and the exflagellation of the male gametocytes

Thick films

Thick blood films provide a rapid method of examining a large amount of blood compared with thin films The technique is of value in finding parasites present at low densities and when large numbers of patients have to be examined It has become a routine method for the detection and differentiation of malaria parasites In addition other infections may be diagnosed such as trypanosomiasis relapsing fever and filariasis A thorough knowledge of the parasites as seen in thin films is required before their diagnosis is attempted in thick films

There are several methods of staining thick films Field s staining method (1941) is a rapid one giving a Romanowsky effect and retaining the haemoglobin in the red cells to provide a background Staining

with dilute Leishman or Giemsa stain removes the haemoglobin from the film leaving only faint remains of the red cells

Thick films are made by placing a drop of blood on a microscope slide towards one end This drop is quickly spread out with a needle so that the hands of a watch may be seen through it at its thickest point It is useful to have a thin margin to the film The film is allowed to dry and should be protected from dust and flies The best results are obtained when it is stained as soon as possible after drying

Field s stain consists of two solutions solution A and solution B These are kept in staining jars with necks wide enough to permit insertion of a microscope slide They are kept stoppered when not in use The thick film is placed in solution A* for 1 second then removed and immediately rinsed gently in clean water for a few seconds until the stain ceases to flow from the film and the glass slide is free from stain The film is then placed in solution B† for 1 second and rinsed by waving gently in clean water for 2 to 3 seconds It is then allowed to dry in a vertical position

Removal of the haemoglobin during staining is preferred by some workers This may be done by staining the thick film with Leishman s stain diluted 1 in 10 with buffer solution and used for half an hour with Giemsa s stain diluted or by diluting Field s stain (A) 1 in 3 with buffer solution and staining for ½ to 1 minute in this solution followed by a more prolonged washing with water before staining with solution B The effect produced is a uniform colourless background to the purple-stained nuclei of the leucocytes red stained platelets and parasites showing blue cytoplasm and red chromatin

Enumeration of parasites

Several methods are available for enumerating parasites In thin films the number of red cells seen infected in counting 1 000 or 10 000 cells compared with the red cell count will give the figure for parasites per cu mm Similarly comparison with the number of leucocytes seen in the film and the total white cell count may be used Sinton

* Solution A

Methylene blue	0 8 g
Azure I	[illegible]
Disodium hydrogen phosphate (anhydrous)	5 0 grams
Potassium dihydrogen phosphate (anhydrous)	6 [illegible]
Distilled water	500

† Solution B

Eosin yellow water soluble	1 0 gram
Disodium hydrogen phosphate (anhydrous)	5 0 grams
Potassium dihydrogen phosphate anhydrous	6 [illegible]
Distilled water	500

Differentiation with 70 per cent alcohol is the critical step in the procedure and the time varies with the thickness of the section and the type of tissue involved The time required is from ¼ to 2 minutes and the tissue is then washed quickly in distilled water as soon as the stain is being removed in noticeable quantities It is then dehydrated and mounted Dehydration is accomplished by passing through a series of xylol-acetone mixtures as alcohol removes the stain The following mixtures are used after the distilled water wash

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PHYSIOLOGY OF THE MALARIA PARASITES

During its sojourn within a red cell lasting 48 hours or 72 hours the trophozoite of the *Plasmodia* of human malaria increases greatly in size and divides into a number of merozoites It depends upon the red cell and the surrounding plasma for nutriment for oxygen and for the disposal of waste metabolites

Studies in the morphology of the parasites have revealed much about th behaviour of the parasite The quartan parasite is slower to develop in its cycle in man and in the mosquito than the other three parasites and remains in the vertebrate host for a much longer period

During their development the trophozoites may show active movement as does *P vivax* and *P falciparum* or this amoeboid movement may be comparatively slight as in *P malariae* and *P ovale* The formation of the vacuole is supposed to be comparable to the development of a food or nutritive vacuole by amoebae The growth of the parasite is comparatively rapid 1 merozoite of *P falciparum* may produce 30 merozoites at the end of 48 hours When the schizont is mature it causes the containing red cell to rupture and release the merozoites into the plasma The method of penetration of fresh red cells by merozoites is unknown and how those of *P vivax* select young red cells preferentially remains a matter for speculation It is known (Stephens 1940) that the surface charge on reticulocytes is different from that on more mature red cells and it is possible that this results in an attraction for vivax merozoites

During its growth in the red cell the trophozoite produces such changes in the containing red cell as enlargement in vivax infections stippling (Schuffner s and Maurer s dots) changes in surface properties so that the cells apparently become sticky in falciparum infections and pallor apparently from the metabolism of the haemoglobin of the red cell

The assimilation of food by the trophozoites raises the question of the ability of the red cell to provide all the materials which are required for growth and division. Nucleoprotein is formed within an erythrocyte which in man contains no nucleus In infection with *P nucleophilum* the parasite is in intimate association with the nucleus of the erythrocyte but appears to cause no change in the morphology of the nucleus Any accessory factors which are not provided by the red cell must reach the parasite from the blood plasma by penetrating the red cell membrane

The failure of sporozoites given in a single dose at one biting session

(1924) mixed malarial blood with an equal volume of a suspension of fowl cells of known concentration and compared the number of parasites with the number of fowl cells in a thick film A further method is to count the number of parasites in a measured volume of blood spread out on a slide and stained by the thick film method (Thomson 1911 b) The measured volume of blood may be spread evenly over a known area on the slide and the parasites counted in a suitable number of microscopic fields of known area This area may be obtained for a given lens-objective combination by the use of a stage micrometer A factor for this combination is derived from the volume of blood used the area over which it is spread and the area of the microscope field The number of parasites per cu mm can then be calculated

A knowledge of the number of parasites circulating in the blood is essential in the control of induced malaria and for studies on epidemiology and in experimental malaria It is valuable also as a guide to the severity of an infection for the clinician

Cultivation of the malaria parasites

Bass and Johns (1912) first reported the successful cultivation of *P vivax* and *P falciparum* They stated that they had observed development for three generations in their cultures This finding that malaria parasites would develop when cultivated *in vitro* was confirmed by Thomson and McLellan (1912) for *P vivax* and by Thomson and Thomson (1913) for *P falciparum* Row described the cultivation *P malariae* in 1917

Many other workers since then have described various modifications of technique but no one has been able to perpetuate a culture of the parasites of human malaria nor has the procedure been developed into a diagnostic method comparable to bacteriological blood cultures

It has been in the cultivation of the parasites of animal malaria that progress has been made Trager (1943 b) reported the survival of *P lophurae* for 16 days in a culture medium containing calcium pantothenate Ball *et al* (1945) used a complicated medium containing inorganic salts and organic substances including vitamins for the cultivation of *P knowlesi* and secured an increase in the number of parasites *in vitro* comparable with that occurring *in vivo* in monkeys Hawking (1944) described the development of the exo-erythrocytic forms of *P gallinaceum* in tissue culture

50 per cent solution to 10 ml of blood and stressed the necessity for anaerobic conditions In 1928 Row described a modified Bass and Johns technique and showed that parasites developed in cultures incubated aerobically

Trager (1941) using *P lophurae* found that cultures were more successful when his inorganic medium was enriched with the following substances red cell extract chick embryo extract glucose or glycogen glutathione and probably yeast extract and a very low concentration of liver extract He later (1943 b) noted improvement in his results when calcium panthothenate as added to the culture medium

Ball *et al* (1945) have described the successful culture of *P knowlesi* using a complex medium of inorganic salts and organic substances including glucose amino acids and numerous vitamins A slow stream of a mixture of 5 per cent CO_2 95 per cent air was bubbled through the medium The cultures were placed on a rocking machine and incubated at 38 5 C They emphasized the importance of para-aminobenzoic acid in the medium

Trager (1946) found that gentle rocking of his cul ures of *P loph irae* under a stream of 5 per cent CO_2 95 per cent air allowed the parasite to multiply in his inorganic medium enriched with red cell extract As yet there have been no comparable results published for the malaria plasmodia of man nor have there been any complete results showing the inorganic and organic requirements of parasites Most workers agree that cultivation at body temperature gives the best results

Another method of approach to the problem of the nutritional requirements of the parasites is to vary the diet of the host and observe the changes produced in the behaviour of the parasite MacDougall (19-7) observed that an increase in the blood sugar concentration in birds infected with *P gallin iceum* brought about a condition favourable for the parasite A decrease in the blood sugar as a result of insulin injections created a condition unfavourable to the parasite

Trager (1943 a) showed that the biotin content of the diet of chicks and ducks influenced the natural susceptibility of these birds to infections with *P loph irae* and *P cathemerium* He found that chicks and ducks deficient in biotin developed much more severe infections with *P lophurae* than did non-deficient control animals He proposed the theory that all malaria parasites require biotin for growth and that for each species of bird parasite there is an optimum range of blood biotin concentration in the host The growth of parasites is slowed if the biotin concentration falls below this range and is inhibited by the direct toxic effect of a biotin concentration above it

to give rise to an infection with *P vivax* in which the trophozoites are all developing in phase is a problem for which no explanation has yet been given Perhaps the trophozoites enter the red cells from the exo-erythrocytic schizonts over a period of time and then commence to develop thus giving rise to asynchronous schizogony James and Shute (1926) described development of synchronous division of parasites with a typical tertian temperature chart as occurring late in primary infections They attributed this to the disappearance of the group of parasites present in the least numbers as vivax malaria tends towards recovery with the disappearance of parasites from the circulating blood

The precipitating factors causing relapses of malaria have not been elucidated It has been usual to attribute these to some change in the environment or bodily constitution of the host but they may well be due to renewed perhaps periodic activity of the exo-erythrocytic parasites The host-parasite relationship has been studied in bird malaria Boyd (1929) found that reversal of the light and dark periods of the host resulted in a reversal in the periodicity of schizogony of *P cathemerium* infections Stauber (1939) showed that variation in the temperature of the environment of the host affected the periodicity in *P cathemerium* and *P relictum* infections Influence of the diet of the host upon the development of the parasites has been reported by Trager (1943 a) and Seeler and Ott (1946) Immune reactions on the part of the host are important in the discussion of the host-parasite relationship and are described elsewhere The difficulty found in establishing human malaria in monkeys and monkey malaria in man indicates either active natural immunity or that the red cells and plasma of the new host do not provide the essential metabolites for the growth of the parasite

Like bacteria trophozoites of the plasmodia may be preserved for long periods in the frozen state Coggleshall (1939) found that monkey blood containing *P knowlesi* and *P inui* frozen rapidly and kept at −76° C remained infective for monkeys for 70 days Brumpt and Dao (1945) however failed to preserve *P gallinaceum* at a temperature of −25° C

The metabolic activities of the malaria plasmodia have been studied in a number of ways The requirements of the parasite in culture were first reported by Bass and Johns in 1912 These two workers stated that glucose or maltose were essential additions to the serum of the culture medium for the growth and division *in vitro* of the *Plasmodia* of human malaria They added glucose in a proportion of 0 1 ml of a

find no clear evidence of phosphorylation of glucose in its metabolism by the parasite

Maier and Coggeshall (1941) showed that the oxygen consumption increases with the development of *P knowlesi* from the ring to the segmenting form They confirmed the utilization of glucose and concluded that this is the substance which meets the normal energy requirements of the parasite Citrate malate fumarate and succinate were not used by the parasite They suggested that glucose was metabolized by the parasites without phosphorylation It is interesting to apply the figures for oxygen consumption given by the last mentioned workers to the malaria of man They found that segmenters of *P cynomolgi* took up 47 0 cu mm of oxygen per hour per 10^8 parasites In man the approximate number of parasites present in the body when 1 per cent of the red cells are infected is 2 500 $\times$ 10^8 and if their oxygen consumption were of the same order as that of *P cynomolgi* this would mean that they require about 2 c c of oxygen per minute compared with a basal oxygen requirement for the whole man of say 220 c c per minute A 10 per cent infection of the red cells would result in consumption of oxygen by the parasites of the order of 20 c c per minute and the haemoglobin of the red cells which are infected with parasites consuming oxygen at this rate is mostly occupied with its supply to the parasite The full amount of haemoglobin in the red cell of a *rhesus* monkey can only combine with enough oxygen to supply the parasite at this stage of development for about 4 minutes In a well-developed parasite much of the haemoglobin has been metabolized by the parasite Admittedly the remaining haemoglobin can absorb oxygen from the plasma and establish an oxygen gradient around the infected red cell

Wendel (1943) stated that approximately one half of the glucose destroyed by *P knowlesi* was converted into lactic acid and the remainder only partly oxidized He suggested that there may be a dependence upon the host for the metabolic processes of the parasite and for the disposal of the end products of metabolism Moulder and Evans (1946) found that chicken erythrocytes infected with *P gallinaceum* produced large amounts of amino nitrogen when incubated in air in the presence of glucose When glucose was absent from the medium much of the amino nitrogen appeared as ammonia Normal chicken erythrocytes formed only a small amount of amino nitrogen or ammonia This finding suggests that *P gallinaceum* can de-aminate amino acids Anaerobiosis strongly inhibited the production of amino nitrogen by infected erythrocytes They prepared cell-free extracts

Although Trager pointed out that the biotin deficiency was unaccompanied by any severe weakening of the animals these results may have been due to an alteration in the resistance of the host rather than to a direct effect on the development of the parasite Seeler and Ott (1946) confirmed the effect upon *P lophurae* parasitaemia in chickens produced by biotin deficiency and reported (1944) that riboflavine deficiency in the chickens resulted in a lowering of the parasitaemia Further if riboflavine was given during the course of the infection it resulted in increased severity as judged by parasite counts Seeler and Ott (1946) also showed that parasite counts in well-nourished chicks infected with *P lophurae* were less than in infected chicks deficient in biotin folic acid and protein

The administration of antimalarial drugs in combination with the feeding of other substances to birds has given some indication of the requirements of bird plasmodia for their development Maier and Riley (1942) reported the inhibition of the action of sulphanilamide on *P gallinaceum* in chickens by the feeding of para-aminobenzoic acid Seeler *et al* (1943) described a similar inhibition of the action of sulphamethyldiazine on *P lophurae* in ducklings also by feeding para-aminobenzoic acid Seeler (1945) stated that the administration of pyridoxine definitely inhibited the action of quinine and atebrin on *P lophurae* and *P cathemerium* in ducklings

In these results there is a similarity to the inhibition by para-aminobenzoic acid and other substances of the action of sulphonamides on bacteria Despite much research on this inhibitory effect the complete mechanism with bacteria has not yet been elucidated The inference is that sulphonamides deprive the malaria parasites of para-aminobenzoic acid or prevent its use by the parasite and as it is an essential metabolite the parasite is destroyed Similarly quinine and atebrin interfere with the parasite s use of pyridoxine and when this substance is administered in excess there is sufficient for the normal metabolism of the parasite and so the effect of these two drugs is modified

Christophers and Fulton (1938) studied the respiration of *P knowlesi* in blood as measured in a Warburg manometer They found that there was a very considerable consumption of oxygen compared with blood containing no parasites An approximately equal output of CO_2 gave a respiratory quotient of 0 91 They stated that serum played little part in providing the requirements of the oxygen uptake and that the parasite was able readily to take oxygen from the oxyhaemoglobin of the red cell Fulton (1939) showed further that glucose laevulose maltose mannose and glycerol were oxidized by *P knowlesi* He could

and illustrated the extracellular position of malaria parasites and the migration of trophozoites and gametocytes from red cell to red cell but this view is not held by modern protozoologists Christophers and Fulton also showed that isolated trophozoites of *P knowlesi* took up oxygen but at a slower rate than when within the red cells

Speck *et al* (1946) freed *P gallinaceum* from the red cells of chickens by means of haemolytic serum and reported that these parasites required various co factors for the oxidation of pyruvate which were not needed when the parasites were studied in intact red cell Free parasites formed appreciable amount of acetate from pyruvate whereas this substance was oxidized almost completely to CO_2 and water by *parasites in intact red cells They concluded that the oxidation of* pyruvate is catalysed by the dicarboxylic acids and involves the tricarboxylic acid cycle and the glucose and lactate are oxidized by the same path as pyruvate

The secretion of a toxin by the malaria parasite has often been suggested This has been supposed to be responsible for the paroxysm when released into the blood by the rupture of the red cells containing mature schizonts While Kudo (1946) states that it is probable that some protozoa secrete poisonous substances through their pseudopodia which possess a paralysing effect upon their prey no toxic secretion of the malaria parasites of man has yet been convincingly reported

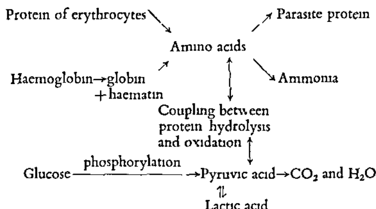
A by-product of the metabolism of haemoglobin by malaria parasites is the malarial pigment haemozoin In *P elongatum* infections of the canary parasites are found in all the types of blood cells (Huff and Bloom 1935) In erythroblasts which have not acquired haemoglobin no pigment is formed but in those members of the red cell series where haemoglobin is present malarial pigment is seen in increasing amounts with increase of haemoglobin in the host cell In the human plasmodia the appearance of the pigment varies with the species of the parasite in *P ovale* it is of a much lighter colour than in the other species Whether or not this difference in appearance is due to chemical differences in composition is unknown

Malarial pigment was known to the ancients as black material in the liver and spleen Meckel (1847) noted black bodies in the blood and spleen and Virchow (1849) first identified the presence of pigment in organs with malarial infection Meckel and Virchow considered the spleen was the site of origin of the pigment Planer (1853) described the pigment in the blood cells and concluded that the pigment originated in the blood When Laveran (1880 a and b) discovered the

of malaria parasites and found that these extracts hydrolysed native haemoglobin at a very slow rate but split denatured globin much more rapidly The production of amino nitrogen from denatured globin in cell-free extracts was not inhibited by anaerobic conditions They suggested that in the intact malaria parasite protein hydrolysis is in some way linked to the oxidative processes

Speck and Evans (1945 a) reported results of the action of cell-free extracts of parasites (*P gallinaceum*) on glucose They stated that glucose was converted to lactic acid by a path similar to that of the phosphorylating enzymes which catalyse the phosphorylation of glucose by adenosine triphosphate the splitting of fructose-1 6-di-phosphate to form 3-phosphoglyceraldehyde and the dismutation between 3-phosphoglyceraldehyde and pyruvic acid

The carbohydrate and protein metabolism of the parasite may then be represented as in the following diagram (adapted from Moulder and Evans 1946)



Despite this apparent reliance of the malaria parasites upon the red cell for its protein metabolism Christophers and Fulton (1939) reported that *P knowlesi* freed from red cells by saponin retained its infectivity for monkeys When the free parasites were injected into the peritoneal cavity of monkeys infection of these animals occurred with some delay in the incubation period compared with controls in which the parasites were still contained in red cells They pointed out however that it was impossible to exclude the presence of segmenting parasites with merozoites in the injected material Were merozoites not present in the injected material the ability of free parasites to enter fresh red cells would have to be considered Lawson (1913 1920) described

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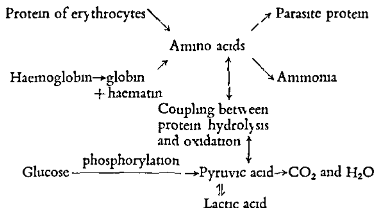
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by the oxyhaemoglobin of the containing red cell and the amount consumed increases with increase in size of the parasite

The protein material of the red cell including the haemoglobin is broken down by the parasite and some of the derivative resynthesized to form parasite protein One waste product—haematin—is deposited as granules in the cytoplasm of the parasite It is possible that some of the materials required for protein synthesis are derived from the plasma by diffusion through the red cell membrane Protein metabolism appears to be linked with the oxidation of glucose

Riboflavine and calcium pantothenate are two substances of known chemical constitution which, with biotin seem to be essential for the normal growth of the *Plasmodia* Para-aminobenzoic acid can neutralize the lethal effect of sulphonamides upon *P knowlesi*—an action similar to that seen with bacteria

The biochemical processes of the malaria parasite which have been described so far are similar to those seen in other organisms with one exception and one which is characteristic of the *Plasmodia* This is the formation of malarial pigment and its deposition in the cytoplasm of the parasite This pigment is not formed by other parasites of the red corpuscle such as the *Babesiae*

Finally it appears certain that there are differences in the biochemical behaviour of the various species of *Plasmodia* which are as definite as the differences in their morphology

MODE OF ACTION OF ANTIMALARIAL DRUGS

When interest was first taken in the method of action of various specific drugs on infecting organisms it was found that these drugs caused a remarkably lethal action on the organism in the body but a like effect was not necessarily seen when a similar concentration of the drug was added to the organism *in vitro*

Bass (1912) added quinine in the high concentration of 1 in 2 000 to a culture of *P falciparum* This prevented growth of the parasites and their death ensued Muhlens and Kirschbaum (1924) added quinine to blood containing parasites (*P vivax*) to give a final concentration of 1 in 5 000 and allowed it to act in an incubator for 5 hours The blood still remained infective for man Lourie (1934 a) added quinine in a similar concentration to blood containing *P cathemerium* and incubated it for 1 hour He found that it caused some delay of infection when the blood was injected into a canary Chopra *et al* (1936) added atebrin to blood containing trophozoites of *P knowlesi* and incubated the

malaria parasite the significance of the pigment in the blood and organs became clearer

Meckel (1847) called malarial pigment melanin and it was for many years considered to be related to the melanin group of pigments Ascoli (1910) suggested that it may be haematin and Brown (1911) as a result of comparison of the solubility and spectroscopic appearances of malarial pigment and haematin considered that malarial pigment was essentially haematin but may contain some impurities Warasi (1927) however *considered that malarial pigment was more* closely allied to an iron-containing melanin than to haematin Sinton and Ghosh (1934 a and b) confirmed the work of Brown and concluded that malarial pigment was identical with haematin Ghosh and Nath (1934) found that the iron carbon and hydrogen content of malarial pigment derived from *P knowlesi* agreed with those of haematin but compared with the latter pigment the amount of nitrogen found was too low They attributed this to experimental error Devine and Fulton (1941) showed that the pigment was not haemin and Morrison and Anderson (1942) also confirmed the pigment to be haematin (ferrihaemic acid) It is now generally accepted as being *haematin derived from the digestion of the haemoglobin of the red* cell and deposited in the form of granules within the parasite Malarial pigment was called haemozoin by Sambon

When the schizont ruptures pigment is released into the plasma and is removed by cells of the reticulo-endothelial system so that it is seen especially in the liver and spleen The pigment of the female gametocytes is readily seen in the oocyst in the mosquito where it has an arrangement and character more or less constant for the particular species of the *Plasmodia* of man In the tissues malarial pigment persists for a long time and Brown (1911) considered that the difficulty in its disposal by the human organism indicated that the production of haematin could not be considered as a normal internal process in the breakdown of haemoglobin Rigdon (1945) considers that the malarial pigment within the endothelial cells may be slowly oxidized *to form haemosiderin which can be used by the host (see Chapter IV)*

Research on the biochemistry of malaria parasites has produced so far an incomplete story The chief features so far elucidated will be summarized *Glucose appears to be the carbohydrate which pro-*vides the energy requirements of the plasmodial cell and it is oxidized probably by phosphorylation to carbon dioxide and water The oxidation of glucose may be incomplete and the parasite may rely upon its host for the excretion of lactic acid The oxygen used is provided

patient but some of the parasites became motile again some hours later when the plasma atebrin concentration dropped Fairley *et al* (1946 a) described the changes seen in *P vivax* after the administration of paludrine Here development ceased at the stage immediately preceding nuclear division Bock and Osterlin (1938–9) showed by photofluoroscopic examination that atebrin was present in the malaria parasites of monkeys when this drug had been used for treatment

Thus changes could readily be produced and often observed in the malaria parasite by antimalarial drugs after their administration to a patient with malaria but changes could not always be produced with similar concentrations of these drugs added to the parasite *in vitro* Black (1946) described the effects of various antimalarial drugs in the form in which they circulated in the blood upon the trophozoites of *P falciparum* developing in cultures *in vitro* The drugs were given in therapeutic doses to healthy donors and the sera from these used for the culture media A direct lethal effect on the parasite by the drugs in this form was found Further the drugs used were observed to act at different points in the developmental cycle of the parasite e g atebrin and quinine acted on the parasite in its early stages of growth whereas paludrine exerted its effect later and prevented the division of chromatin to form schizonts (see Table III) This suggests that

TABLE III

Drug in Serum of Culture Medium	*Forms Developing in Culture*						
	R	*A*	*Ps*	*Es*	*Ds*	*Ms*	*R*
Nil (Control)	+	+	+	+	+	+	+
Quinine	+	+	+	—	—	—	—
Atebrin	+	+	—	—	—	—	—
Resochin	+	+	—	—	—	—	—
Santochin	+	+	—	—	—	—	—
Paludrine	+	+	+	+	—	—	—
M 4430	+	+	+	+	—	—	—
Sulphadiazine	+	+	+	+	+	±	±
Sulphamerazine	+	+	+	+	+	±	±

TABLE 3—Res lts obt in d when rious ant malarial drug n the f m in whi h th y ircul te in th body re allo d to a t on *P f lcip r m* (N w Gui trains) d elop ng in culture N l R n g f rm A moeb d f rms, P p esch o ts E early h ont Ds sch z nts w h some d is on f ch omatin Ms m ture sch zo ts R ri g of se d ge e t n + fo m dev l p ng in cultu e — n f m seen (Co tructed f m Bl k 1946)

preparation for 24 hours The blood was then inoculated into a group of monkeys of which one developed malaria and two did not The concentration of atebrin in this experiment (20 000 γ per litre) was much higher than is ever obtained with therapeutic doses of this drug Hewitt and Richardson (1943) added drugs either in the form of a powder or in solution to blood infected with *P lophurae* and incubated their preparations at 6° C They found that typical degenerative changes occurred in the parasites when plasmoquine was used These changes were similar to those seen when infected ducks were treated with plasmoquine They failed to produce degenerative changes in the parasites with quinine and atebrin Tonkin (1946) added various antimalarial drugs to tissue cultures of the exo-erythrocytic forms of *P gallinaceum* Some of the drugs e g paludrine had no effect but inhibition of parasite growth was obtained with several drugs, including some of the sulphonamides and *p*-anisylguanidine nitrate

Studies have been made on the depression of respiration of the parasites by antimalarial drugs added *in vitro* to blood containing plasmodia Fulton and Christophers (1938) found that quinine and atebrin caused inhibition of respiration of *P knowlesi* Coggeshall (1940) showed that the addition of sulphonamide in concentrations less than those necessary to effect a cure in monkeys almost completely inhibited the respiration of *P knowlesi* The same concentrations of the drug had no apparent effect on the respiration of *P inui* a parasite which is much less sensitive *in vivo* to sulphonilamide than is *P knowlesi* Nevertheless Coggeshall and Maier (1941) reported that the results obtained with this method did not always agree with the observed therapeutic effect of the drugs when they were used *in vivo*

Observations have been made on the changes in morphology and development of parasites after the administration of antimalarial drugs to patients suffering from malaria Wenyon (1926) described a decrease in the size of *P vivax* schizonts and a reduction of the number of merozoites formed With *P cathemerium* in canaries Lourie (1934) found that quinine caused a retardation of growth of the parasite and the formation of a smaller number of merozoites James (1934 a) demonstrated pigmentary and cytoplasmic changes produced by the action of atebrin on *P vivax* and *P malariae* and Huhne (1942) described the degenerative changes seen in the parasites when atebrin was given to patients with falciparum malaria Young *et al* (1943) reported that thiobismol had an inhibitory effect on half-grown trophozoites of *P vivax* Trager *et al* (1945) stated that *P vivax* lost its amoeboid activity an hour after an intra-muscular injection of atebrin into a

was administered during the sulphonamide treatment of birds infected with *P gallinaceum* and *P lophurae* Para aminobenzoic acid had no effect on the action of quinine and atebrin upon *P gallinaceum* Tonkin (1946) reported that the inhibitory effect of sulphathiazole in cultures of the exo-erythrocytic forms of *P gallinaceum* was neutralized by para-aminobenzoic acid Ball (1946) reported antagonism between para aminobenzoic acid and sulphadiazine in cultures of *P knowlesi* In these last results there is a close resemblance to the inhibition of sulphonamide action upon bacteria caused by para-aminobenzoic acid

In a series of studies of the biochemistry of *P gallinaceum* by Speck Moulder and Evans (Speck and Evans 1945 a and b Moulder and Evans 1946 Speck Moulder and Evans 1946) quinine and atebrin were found to inhibit the glycolytic enzymes of the parasite although the concentration required was relatively high Low concentrations of these drugs were found to inhibit strongly the production of amino-nitrogen by parasitized erythrocytes They suggested that quinine and atebrin acted by inhibiting the oxidation of the pyruvic acid produced by the parasite to carbon dioxide and water Bovarnick *et al* (1946 a and b) found that atebrin inhibited the oxygen consumption of *P lophurae* which had been released from erythrocytes by saponin This inhibition was much more marked if the parasites had previously been deprived of substrate and could be partially neutralized by the addition of adenosine triphosphate and adenylic acid They concluded that the action of atebrin on the parasites is due to interference with some phosphorylation reactions which are necessary before glucose can be utilized Madinaveitia (1946) showed that some antimalarial drugs were antagonistic for riboflavin in the growth of *Lactobacillus casei* Curd and Rose (1946) reported that paludrine does not show this antagonism for riboflavine and suggested that the antimalarial activity of paludrine may be connected in some way with an interference with the porphyrin metabolism of the parasite as this drug forms with metals such as copper a complex compound resembling protoporphyrin

The report by Trager (1943 b) that calcium pantothenate was a growth factor for *P lophurae* led to the investigation of related compounds to determine if any of these had antimalarial activity Mead *et al* (1946) stated that a derivative of *d* pantoyltaurine showed definite activity in avian malaria Marshall (1946) reported the use for the treatment of malaria of a compound prepared by Wooley (1944) and shown by him to be a pantothenate antagonist for bacteria This compound—pantothenone—was found to be as active as quinine against

the immediate site of action of these drugs is different though the action is a direct one on the parasite itself atebrin and quinine would appear to antagonize the activities of the cell concerned with its early growth whereas paludrine interferes with its nuclear division Hawking (1947) reported the anti-plasmodial action of paludrine upon the exo-erythrocytic forms of *P gallinaceum* when serum from a fowl treated with this drug was added to cultures of these parasites Wendel (1946) stated that certain drugs in the serum after administration to the host depressed the respiration of parasites *in vitro*

In these last results the drug used to act on the parasites was in the form in which it circulates in the body In the case of atebrin given by intramuscular injection the serum for the cultures was obtained from blood taken only 15 minutes after the injection and yet it was as effective as serum with similar concentrations of atebrin which had been ingested Black (1946) pointed out that if atebrin must be in a combined or altered form before it can act on parasites alimentation is not necessary for this modification of the drug and the change occurs within 15 minutes of its intramuscular injection In the case of paludrine Hawking (1947) suggested that the drug is activated in the body

The exact manner of the direct lethal action of the drugs on the parasite is a biochemical problem The existence of complex enzyme systems in the single cell of a malaria parasite has already been discussed The normal functioning of these systems is presumably necessary for its survival and growth Thus there are many vulnerable points for an attack on the malaria parasite or indeed on any cell Walker (1928) showed that arsenic exerts its toxic effect on living cells by attacking some essential sulphydryl component in the enzyme system of the pyruvate cycle Henry (1944) concluded that the sulphonamides destroy bacteria by inhibiting one or more of their respiratory systems The divers antimalarial drugs may act on the various enzyme systems essential for the growth and division of the plasmodial cell

There is already evidence that this is the method by which these drugs act on the parasite Trager (1943 b) showed that when calcium pantothenate was added to his cultures of *P lophurae* the parasites grew for longer periods than in its absence Seeler (1945) reported that the administration of pyridoxine inhibited the action of quinine and atebrin on *P lophurae* and *P cathemerium* in ducklings Inhibition of the action of the sulphonamides on malaria parasites has been reported by Maier and Riley (1942) and Seeler *et al* (1943) when para-aminobenzoic acid

causes degenerative changes in the chromatin and cytoplasm of gametocytes as described and illustrated by Mollow (1928) in falciparum malaria

Paludrine in therapeutic doses affects neither the morphology nor the number of gametocytes already formed in infection with *P falciparum* and *P vivax* (Fairley *et al* 1946 a) These workers also showed that the effect of paludrine on the gametocytes is seen in the gut of the mosquito If mosquitoes are fed on gametocyte carriers who have recently had paludrine development may proceed up to the oocyst stage but owing to the persistent effects of the drug all oocysts die In mosquitoes which had engorged partially on a normal person taking 100 mgm of paludrine daily and allowed to complete their feed on a falciparum gametocyte carrier oocysts formed in the gut but died without growing and sporozoites completely failed to reach the salivary glands The effect in the oocyst appears to be much the same as the effect produced by paludrine on the developing trophozoite i e inhibition of nuclear division in this case preventing the formation of sporozoites and causing death of the oocyst Missiroli and Mosna (1938) reported a similar effect with the action of minute doses of Cilional (Certuna) on the gametocytes of *P falciparum* This drug in large doses destroys the gametocytes smaller ones prevent exflagellation and though minute doses cannot prevent the fertilization of the gametes inside the mosquito yet they can and do hinder the further development of the oocysts

The acquisition of resistance to paludrine by *P gallinaceum* has been described by Bishop and Birkett (1947) and Williamson Bertram and Lourie (1947) The parasites were exposed to non-sterilizing concentrations of this drug during serial passage through chickens secured by trophozoite inoculation The gametocytes were able to infect *Aedes aegypti* and the sporozoites formed when injected into chicks produced infections which were equally resistant to paludrine Fairley *et al* (1946 b) describing atebrin-resistant strains of *P falciparum* found in the Aitape Wewak area of New Guinea suggested that this resistance may have been a result of prolonged suboptimal dosage of the drug Successive passages of one of the strains through mos quitoes suggested that it had lost its atebrin resistance Bishop and Birkett (1947) and Williamson Bertram and Lourie (1947) reported that with *P gallinaceum* they had been unable to produce atebrin-resistant strains with a period of exposure of these parasites to the drug of 6 months to 2 years

The acquisition of drug resistance by bacteria is well known but

P gallinaceum in the chick This activity is antagonized by pantothenic acid There is, apparently competition between these two substances for an enzyme necessary for the use of pantothenic acid by the parasite

A specific combination of the antimalarial drug with some particular component of the parasite is indicated when the therapeutic effects of an additive series derived from one compound are studied Findlay (1939) discussed the expansion of one side chain of an antimalarial drug and detailed the chemotherapeutic indices of the compounds of the series formed The antimalarial properties of this series of drugs depended on the presence of an odd number of carbon atoms in this side chain This recalls the original theory of Ehrlich when he explained the action of salvarsan certain side chains of the drug had a selective chemical affinity for certain side chains in the protoplasm of the spirochaete Support for this theory is found in the differences in susceptibility of various species of malaria parasites to antimalarial drugs Bishop (194-) stated that there are drugs such as Paludex which are more effective in treatment of the malaria of man than in avian malaria Green (19-9 b) reported on Dimenplasmin *which although ineffective in the treatment of human malaria is* highly effective against avian malaria Amongst the *Plasmodia* of human malaria there is also this difference in susceptibility to antimalarial drugs Plasmoquine is a moderately effective trophozoiticide in vivax malaria but it is inefficient as such in falciparum infections Similarly Young *et al* (1943) found that thiobismol was active against *P vivax* but had no effect on *P falciparum* Thus there is often a species difference in the reaction of plasmodia to antimalarial drugs Further Lourie (1934 b) showed that two strains of *P relictum* differed in their response to identical amounts of quinine when present in canaries This variation in response to drugs is not dependent upon differences in the hosts but is probably due to some subtle differences in the biochemical activities of the parasites

Although much has been elucidated about the mode of action of the antimalarial drugs there is as yet no explanation for their selective action upon the *Plasmodia*—no biochemical process has been described for the parasites which has not a parallel in the host apart from the formation of malarial pigment

Little is known about the mode of action of gametocidal drugs beyond the results obtained from direct observation of the effects produced in the morphology of the gametocytes in the vertebrate host and of their subsequent behaviour in the mosquito Plasmoquine

CHAPTER III

THE BLOOD CELLS

ANAEMIA. ERYTHROCYTE COUNTS THE BLOOD PICTURE RETICULOCYTES DIFFERENTIAL INVASION OF RED CELLS CHANGES IN RED CELL APPEARANCE CHANGES IN RED CELL PIGMENT HAEMOLYSIS Phagocytosis — mechanism of haemolysis SEDIMENTATION RATE COAGULATION TIME LEUCOCYTES AND PHAGOCYTES

ANAEMIA

THE maturation of the schizont with the liberation of merozoites into the plasma leads to the destruction of the parasited erythrocyte Through some process not yet fully understood red cells which are not actively invaded by plasmodia are also destroyed in large numbers According to Garin (1930) the number of red cells destroyed in a typical paroxysm is about two hundred thousand Howie (194-) found in *P falciparum* infections that the fall was even greater amounting to five hundred thousand cells per cu mm in some cases with an associated fall of haemoglobin concentration of 10 per cent The reduction in oxygen carrying capacity of the blood resulting from such losses of erythrocytes affects the erythropoietic centres of the body and brings about changes in the rate of production of new cells and possibly (Thonnard-Neumann) in the rate of discharge of new cells into the general circulation Other factors such as disturbances of vascular flow and stasis of cells in the small blood vessels probably enhance this process

The result of these phenomena is the development of anaemia The severity of this varies from patient to patient and depends largely upon the species of invading plasmodium the degree of invasion of erythrocytes and the duration and severity of the infection In acute active cases the reduction in red cells is associated with a roughly proportional reduction in haemoglobin concentration i e the anaemia is usually of the simple type In long-standing infections the characteristics of the anaemia may be modified to some extent by accompanying changes in the bone marrow

Anaemia is usually most pronounced in *P falciparum* infections in humans and in *P knowlesi* infections in monkeys In acute severe *P falciparum* infections it often develops extremely rapidly and progresses sometimes in a series of crises during which the red cell count may fall by as much as a million cells per cu mm in the course of

bacteria multiply by asexual division In the case of the resistance to paludrine the gametocytes appear to have been modified so that they were able to pass on this resistance to the next asexual generation An alternative explanation is that the apparent acquisition of resistance is due to the survival and multiplication of a certain few parasites which were naturally resistant to paludrine i e the original strain was not absolutely homogeneous with regard to its response to paludrine This theory presupposes that gametocytes formed by these asexual parasites were also resistant to paludrine Whatever the explanation— and it is apparent that one cannot definitely be formulated at this stage—these paludrine-resistant parasites appear to differ in their biochemical behaviour from those parasites which show the usual reaction to paludrine

field derived from both natural and induced *P vivax* infections may be quoted. These authors reported red cell counts varying from 2.2 to 4.9 million per cu mm, averaging 3.99 million per cu mm, with haemoglobin concentrations from 40 to 90 per cent, with an average of 76 per cent. Colour indexes in these cases averaged 0.95.

The anaemia seen in both *P malariae* and *P ovale* infections is generally mild and of the order of that seen in *P vivax* infections. Severe *P malariae* infections have been reported, however, in which anaemia was pronounced.

THE BLOOD PICTURE

In very severe acute primary infections there may be little change in the appearance of the erythrocytes. Where, however, the disease has progressed for some time or has reached a chronic stage or is relapsing, the blood picture presents characteristics superficially resembling those seen in pernicious anaemia. There is poikilocytosis and anisocytosis, polychromasia and basophilic stippling. Both parasitized and unparasitized cells may show signs of change.

The anisocytosis is associated with both small and large red cells. Fairley and Bromfield (1933) did not find any appreciable increase in the diameter of the red cells in their cases. Thus in 14 cases of *P falciparum* infection halometer measurements gave a mean of 7.5 μ, with a range of 7.2 to 7.8 μ for the erythrocyte diameter, and in the same number of cases of *P vivax* malaria the mean diameter was 7.2 μ, with a range of 6.8 to 7.8 μ. In one case of *P ovale* infection the average red cell diameter was also 7.2 μ. Fairley, Bromfield, Foy and Kondi (1938) drew Price Jones curves in three moderately anaemic patients with severe malaria (parasites present in the blood). They found the curves fell within the ideal curves for the smallest and largest mean diameter, the average diameter being 7.35 μ. Nevertheless some degree of megalocytosis has been reported by many other authors both in naturally acquired and artificially induced malaria. For instance Suarez and Costa Mandry (1934) found that in chronic malaria the anaemia was hyperchromic with a tendency to macrocytosis. Thonnard-Neumann (1944) made careful estimations of the diameter of red blood corpuscles in the progress of induced *P vivax* infection in syphilitic patients before, during and after treatment with malaria. He found that during a malarial attack there was both an increase in the mean corpuscular diameter and a rise in the colour index, both these values returning to normal rapidly after treatment. At the com-

24 hours In such cases there is usually accompanying haemoglobinuria and the general clinical picture of the blackwater fever syndrome but very rapid and progressive reduction in erythrocytes may occur in the absence of haemoglobinuria so that in the course of a few days the total red cell count may fall below a million per cu mm The anaemia of *P falciparum* infection in its degree and rapidity of development in active cases resembles in many ways that of *P knowlesi* infection in monkeys Fairley (1934) however has pointed out that the critical fall in the number of red cells occurs in untreated *P knowlesi* infections in the matter of a few days and is always associated with progressive increase in the number of parasites In *P falciparum* infections on the other hand the invasion of erythrocytes in cases exhibiting severe haemolytic anaemia is not always very great and severe anaemia may not develop in infections until they have gone on for some time

The red cell counts observed in cases of *P falciparum* malaria seen under ordinary circumstances in the tropics and in England are seldom very low They may even be within normal limits Suarez and Costa Mandry (1934) for instance report a red cell count of five million and a haemoglobin concentration of 110 per cent in an acute case Fairley and Bromfield found that in 30 cases of malignant tertian malaria examined in England the red cell count varied between 1 8 and 5 2 million per cu mm The haemoglobin concentration varied between 32 and 102 per cent with an average of 73 per cent and the colour index averaged just less than one Figures such as these have often been reported but much lower figures for both erythrocyte count and haemoglobin concentration e g of the order of 1 0 million cells per cu mm and 30 per cent haemoglobin or less are not infrequently encountered in individual cases Nelson Jones states that in his patients in the Gold Coast no great fall took place in the blood cells except in severe cases of haemolytic anaemia in which a reduction of the erythrocyte count of the order of a million cells per cu mm might occur during a single haemolytic crisis

In *P vivax* infections the degree of anaemia is usually less than that seen in *P falciparum* malaria Occasionally however especially in children the anaemia may be severe and in exceptional cases approach that seen in *P falciparum* infections Thus Carducci (1907) records a red cell count of 0 44 million cells per cu mm and Amy (1934) one of 0 56 million cells per cu mm in fatal cases of *P vivax* malaria As an example of the degree of anaemia usually seen in patients suffering from benign tertian malaria the figures of Fairley and Brom-

in the peripheral blood in one fatal case of *P falciparum* infection and in a case of *P vivax* infection during the ninth successive rigor

RETICULOCYTES

The production of reticulocytes and their appearance in increased numbers in the circulation may influence to some extent the mean diameter of the red cells seen in the peripheral blood The presence of reticulocytes in the blood may also account for the polychromasia mentioned by many authors since it has been pointed out by Whitby and Britton (1946) that such irregular staining of red cells is related to their immaturity

Reticulocyte response in malaria infections has been described by many authors but as far as the reticulocyte concentration during an actual attack is concerned the findings of workers are often at variance Fairley (1934) and Fairley and Bromfield (1933) found that there were between 0 6 and 7 8 per cent of reticulocytes present in their malaria cases (both natural and induced cases of *P falciparum* and *P vivax* infections) before treatment In one case the reticulocyte count was as high as 10 per cent on the second day of treatment After the administration of quinine and immediately following the disappearance of parasites and fever there was in all cases an increase in reticulocytes to an average maximum of 10 per cent and in one case to 23 per cent The maximum responses in all cases occurred 6 to 10 days after initiation of treatment It was found that the degree of reticulocytosis in these cases was directly related to the degree of anaemia present For example in a case in which the red cell count varied from 5 to 4 1 million cells per cu mm the maximum reticulocyte count recorded was 9 4 per cent whereas in another case where the blood count varied from 2 to 1 1 million cells per cu mm the maximum reticulocyte count was 29 3 per cent

Benhamou (1933) reported that in mild malarial anaemias where the blood count ranged from 3 5 to 4 million cells per cu mm the reticulocyte concentration varied from 3 to 5 per cent In more severe anaemias the reticulocyte percentage increased in one case reaching as much as 19 per cent This latter figure approximates closely to that recorded by Yang and Berglund (19_9) who found a reticulocytosis of _3 per cent in an untreated tertian malaria case with an erythrocyte count of 1 million per cu mm These authors regard such high reticulocytosis as evidence of bone marrow regeneration resulting from haemolysis High reticulocyte counts have also

mencement of treatment in these patients the mean red blood cell count was 4 5 million per cu mm and the mean haemoglobin 80 per cent At the end of the active clinical malaria (cases were allowed 10 paroxysms) the mean red cell count was 2 7 million per cu mm and the haemoglobin 60 per cent The colour index in 15 out of 22 *P vivax* infections was less than one on the first day of infection and in 19 was greater than one at the beginning of treatment after treatment 20 cases had colour indices of less than one In 10 cases Price-Jones curves were drawn The mean cell diameters were found to be 7 3 μ 7 7 μ and 7 3 μ at the start at the height of infection and shortly after cure respectively

Thonnard-Neumann was dealing with induced malaria but similar results have been reported under field conditions Thus Seelig and Hemming (1944) noted megalocytic anaemia as a sequel to chronic malignant tertian malaria in Indian troops These authors describe the clinical picture as pernicious anaemia without involvement of nerve tissue The significance of their results is perhaps not very great in view of the possibility of involvement of other factors such as malnutrition However such findings are in agreement in general with those of many other observers Bianchi (1940) for instance has reported that in acute malignant tertian malaria in Sardinia macrocytes are constantly present in the peripheral blood and that the colour index is greater than one Schretzenmayr (1938) has stressed the similarity between the blood picture in malarial anaemia and pernicious anaemia and also reported high colour indexes in the former The similarity between pernicious anaemia and malarial anaemia is however a very superficial one and is related more to the picture seen in the peripheral blood than to changes in the bone marrow It is generally agreed that macrocytes may be present in malarial anaemia especially in its more chronic stages and that nucleated red cells are uncommon and cells resembling megaloblasts extremely rare Normoblasts have been reported in the peripheral blood and would be anticipated in pronounced anaemia of malarial origin in which the predominant response of the bone marrow is normoblastic a compensatory hypertrophy resulting from the continued haemolysis

Megaloblasts when present in the peripheral blood according to Fairley and Bromfield arise from hyperstimulation of the marrow probably in response to an anoxaemia consequent on anaemia True megaloblastic response has however been reported and Bianchi (1940) believes that the bone marrow is sometimes directly involved in malaria Thonnard-Neumann reports the presence of megaloblasts

the peripheral blood The author therefore suggested that there was an inverse relationship between the presence of parasites in the peripheral blood and the height of the reticulocyte count Further investigations involving repeated examinations of the bone marrow showed that during the active stage of malaria there was no restriction in the production of reticulocytes in the bone marrow In fact reticulocytes increased in the bone marrow until in some cases it contained as many as eight times the proportion of reticulocytes present in the peripheral blood (the normal relations are according to Neumann about three to one) Following treatment of the malaria the rapid increase of reticulocytes in the peripheral blood was associated with a further increase of production in the marrow Upon reduction of parasitaemia by appropriate treatment the reticulocytes formed in the marrow were apparently flushed out into the circulation giving rise to a reticulocytosis in the peripheral blood Thonnard-Neumann therefore considers that in some way peripheral parasitaemia gives rise to a damming up of the newly formed reticulocytes in the bone marrow and that the anaemia produced by the infection is to some extent thereby increased in degree since there is little replacement by reticulocytes of the cells destroyed by parasites and by haemolytic processes initiated by the disease The suggestion that the reticulocyte crisis in the blood following specific therapy can be correlated with the removal of a barrier in the bone marrow which obstructs the release of reticulocytes has also recently been made by Gramiccia (1945)

DIFFERENTIAL INVASION OF RED CELLS

It is generally accepted that in *P vivax* infections the invaded red cells become enlarged during the invasion of the parasite Wenyon (1926) stated that the fully grown schizont was 10 to 11 μ in diameter and Craig (1937) found that the diameter of the segmenting schizont varied as much as from 9 to 15 μ According to Wenyon expressing a widely held view the invaded red cell increases in diameter as the parasite develops in it and in confirmation of this Wilson and Wilson (1935) found that the relative mean diameter of the infected cell was 9 1 μ Reckoning the normal diameter of the red cell as about 7 6 μ this means that the infected red cell had increased on the average by 1 2 times its normal diameter Increase in size of red cells after invasion by parasites has also been observed in *in vitro* cultures

It is possible however that simple enlargement of the red cell in order to accommodate the growing plasmodium is not the only factor

been noted by other authors during the course of untreated malaria in both humans and monkeys particularly in *P knowlesi* infections in monkeys (*M mulatta*) Such big counts have been recorded as the rule only in the later stages of severe infections Thus Malamos reported a reticulocyte count of 35 per cent on the penultimate day of a fatal *P knowlesi* infection in a monkey (*M mulatta*) Bianchi (1940) however in a series of eight cases of malignant tertian malaria in Sardinia found a reticulocytosis of between 4 and 12 5 per cent

The reticulocyte count varies from day to day in individual cases Kitchen (1939) for instance has recorded reticulocyte rates varying from 1 5 to 7 5 per cent in a case of induced *P falciparum* infection 0 3 to 9 3 per cent in another which was untreated and 0 2 to 1 5 per cent in a third which received a single dose of quinine In two cases of induced *P malariae* infection the reticulocytes ranged from 0 1 to 3 5 and 0 6 to 4 5 per cent respectively The first of these *P malariae* cases was fatal and it is interesting to note that in this case in which the parasite density reached the unparalleled concentration (for this species) of 71 000 parasites per cu mm the reticulocyte response in the last days of the illness did not exceed 3 per cent

Most authors agree that in both human and monkey malaria a big increase of reticulocytes occurs during specific drug treatment The rise in reticulocytes observed under these conditions is usually higher than that seen in untreated cases except towards the end of a severe infection Fairley s figures in this connection have already been quoted for human malaria This author reports a concentration of reticulocytes of 35 per cent occurring in a monkey with *P knowlesi* infection seven days after treatment was commenced Dahne (1944) has noted similar increases in reticulocytes after treatment of human malaria with atebrin Thonnard-Neumann has investigated this phenomenon in detail in induced *P vivax* *P malariae* and *P falciparum* infections in syphilitics Before infection the reticulocyte counts of his patients varied from 0 1 to 1 4 per cent During the active stage of the disease the reticulocyte count was not raised in any case above 2 5 per cent with an average of 0 6 per cent for 20 cases A rise in reticulocyte concentration was noted in all cases after specific therapy The mean maximum reached after treatment was 6 5 per cent Thonnard-Neumann drew attention to the relation between the existence of parasitaemia and reticulocytosis Some of his cases showed a double wave of reticulocytes during antimalarial treatment and it was discovered that the fall in reticulocytes observed on these occasions always corresponded with the appearance of parasites in the cells of

cell and argued from this that invasion could not have occurred only in the reticulocyte stage which according to various authors lasts 12 to 24 hours Malamos (1937) studied the comparative invasion rates in mature and young red cells in the malaria of monkeys (*P knowlesi* and *P cynomolgi*) and man (*P vivax* and *P falciparum*) In all infections he found only a small proportion of plasmodia in the reticulocytes In one case of *P falciparum* malaria there was no infection of reticulocytes He pointed out however that when they did occur in reticulocytes the plasmodia existed mostly in young forms except in *P knowlesi* infections where they were sometimes in the half developed state It was possible therefore that although he was unable to confirm Hungst s changes in reticulum staining the reticulum of some cells might have suffered damage during the growth of the plasmodia Malamos found that on the whole the invasion of reticulocytes ran roughly parallel to the incidence of reticulocytosis

Most observers in investigating this problem have relied on the examination of blood films taken at regular intervals during the course of an infection but as the argument really depends on what cells are invaded by merozoites the correct point of investigation should be the time immediately following the completion of schizogony Eaton had pointed this out and in his single case found that multiple infection of cells mostly reticulocytes occurred to the greatest degree within 2 to 4 hours of sporulation Hegner (1938) followed up this observation by examining preparations from one infection each of *P vivax* *P falciparum* *P malariae* and *P knowlesi* made during the period of segmentation He found that 89 per cent of the young rings of *P vivax* invaded reticulocytes the relative frequency of invasion being 1 29. 1 compared with mature cells He also observed that the reticulocytes containing the small rings were as large as those containing half-grown parasites from which he concluded in agreement with Craig that the cells in *P vivax* infections were already large when invaded In *P falciparum* and *P malariae* malaria 96 per cent and 89 per cent respectively of the freshly invaded cells were mature The comparative rates of infection with these last two species were 14 1 and 10 1 for reticulocytes compared with nature cells *P knowlesi* invaded both types of cell equally 99 per cent of the rings being found in the mature cells Kitchen (1938 1939) investigated the problem further in induced *P vivax* *P falciparum* and *P malariae* infections He found over a long series of studies that in two cases of *P vivax* infections the frequency of parasitization of reticulocytes was always greater than that of mature cells the actual degree of difference depending on

concerned in the size of the invaded cell for it has been pointed out by Craig (1926) amongst others that the infected red cell may be larger than normal even when the invading plasmodium is in a very early stage of development This observation has not been generally confirmed but where the point has been specifically examined some evidence has been produced to show that many of the red cells recently invaded particularly in *P vivax* infections are of large diameter and it has been suggested that the explanation of this is that the plasmodium preferentially invades the younger red cells which are usually of greater diameter than more mature cells

Such differential invasions of young cells was suggested by Craik (1920) who in examining the blood in *P vivax* infections found that the parasites although few in total number were most often observed in young red cells and that where multiple infection of cells occurred the red cells concerned were the youngest corpuscles

P falciparum trophozoites were subsequently reported in nucleated red cells in the bone marrow and in the peripheral blood by Thomson and Robinson (1929) and Tobb (1930) but the matter did not receive much attention until Eaton (1934) pointed out the prevalence of reticulocyte infection in the blood of a case of unspecified malaria with quotidian fever Eaton suggested as a working hypothesis that red cells were susceptible to invasion by plasmodia only when they were in the reticulocyte phase and that as a corollary anything increasing the number of reticulocytes would favour the spread of the disease His provocative contention had the desired effect and in the course of following years a good deal of work careful and otherwise was directed towards investigating the invasion of reticulocytes in malaria Jacobsthal (1936) claimed in a note unsupported by detailed evidence that in fresh *P vivax* and *P falciparum* infections there is a preponderate invasion of reticulocytes He suggested that at least 90 to 98 per cent of the invaded cells were reticulocytes and that in the infected cells there were demonstrable changes in the reticulum He pointed out that such heavy invasion of reticulocytes might affect the development of anaemia since the old cells when destroyed were not easily replaced (see Thonnard-Neumann)

Baserga (1937) failed to confirm Jacobsthal s findings and other workers soon observed differences between the behaviour of various plasmodia Thus Shushnan Blitz and Adams (1937) recorded that reticulocytes were selectively invaded by *P vivax* whereas reticulocytes and mature cells were invaded with equal facility by *P falciparum* Corradetti (1937) observed large and small parasites in the same red

than in the bone marrow in *P vivax* infections Beltran and Sandoval (1945) however reported similar infection rates in blood and marrow After making due allowance for this what is found to occur in the peripheral blood is probably a fair indication of the fundamental differences existing between the three plasmodia in regard to their invasive powers of the host s red cells. Such differences in the invasive tendencies of the human plasmodia may explain to some extent the degree of parasitaemia met in the various forms of malaria For example the predilection of *P vivax* for reticulocytes which are normally in low concentrations might limit the spread of this plasmodium whereas the apparent indifference of *P falciparum* to the age of the erythrocytes would place no such natural limits upon its dispersion amongst the red cells. The limitation of parasitic densities of infection with *P malariae* might similarly be partly dependent upon its preference for the more mature red cells its much slower development in comparison with *P falciparum* being due to its longer asexual cycle and production of relatively few merozoites at sporulation The degree of development

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the time of examination of the blood Thus in one case the relative rates of invasion varied from 19 to 1 500 times greater for reticulocytes than for mature cells and in the other case from 7 to 1 250 times greater In both cases the total rate of infection of cells was less than 1 per cent and the rate of infection of reticulocytes was never greater than 86 per cent In *P falciparum* infections the total number of mature cells parasitized was always greater than the number of reticulocytes invaded Mature cells were invaded on all occasions during which blood was examined Reticulocytes were found invaded in only 28 out of 40 examinations From this Kitchen concluded that *P falciparum* was indifferent to the age of the erythrocyte invaded In *P malariae* infections the plasmodia invaded proportionately many more mature than immature cells thus reticulocytes were found to be invaded in only 3 out of 18 observations

Recently Beltran and Sandoval (1945) found that in induced *P vivax* malaria in general paralytics 15 to 90 (average 41 8) per cent of the early ring forms of the parasite were found in reticulocytes indicating a predilection on the part of the merozoites for reticulocytes Ferrebee Gibson and Peacock (1946) have also produced evidence of differential invasion of reticulocytes in *P vivax* infection They studied the distribution of radioactive iron incorporated in the haemoglobin molecule in a case of *P vivax* malaria and observed that whole blood from a patient who had been given intravenous radioactive iron 24 hours previously was much less radioactive than concentrates of infected cells (Ferrebee and Geiman 1946) Since radioactive haemoglobin is found only in newly-formed red cells under the conditions of the experiment (24 hours after intravenous injection of radioactive iron) they infer that the parasites preferentially invade young red cells

Although there is some disagreement among workers in this field it seems established on the balance of evidence that in *P vivax* infections the plasmodia show a predilection for invading immature red cells whereas in *P falciparum* infections both mature and immature cells are invaded about equally and in *P malariae* infections the plasmodia invade mainly the mature erythrocytes

In assessing the value of these observations however it must be pointed out that the differential rates of infection of cells measured in pheripheral blood may not necessarily indicate exactly those rates obtaining in the bone marrow and other organs where maximum sporulation e g in *P falciparum* is taking place In this connection it is interesting to record that Thonnard-Neumann found that the rate of infection of reticulocytes was higher in the peripheral blood

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CHANGES IN RED CELL APPEARANCE

Minor changes in the size and shape of invaded erythrocytes such as the enlargement of the cells in *P vivax* infections are also seen in *P falciparum* and *P ovale* malaria In *P malariae* infections the invaded erythrocytes are not usually altered in appearance Erythrocytes invaded by *P falciparum* appear normal in the early stages of development of the parasites but as the disease progresses they tend to become irregular and distorted in shape and sometimes reduced in size and appear brassy in stained films The changes in shape of invaded cells in *P ovale* malaria are characteristic of the infection In the early stages of invasion when the parasite is in the young ring forms red cells are

not uncommonly oval with irregular margins—*fimbriated* (Stephens 1922) The red cells are not enlarged at this stage but later as the parasite develops to the stage of segmentation they may be sometimes slightly increased in diameter more frequently however remaining unchanged in size

In preparations of infected blood certain changes in the staining of invaded red cells have been observed in various infections In *P vivax* malaria the so-called Schuffner s dots appear when the trophozoite has become mature and are most evident during the schizont stage Schuffner s dots show up as small or large reddish dots scattered evenly over the affected red cell They are never present in uninvaded erythrocytes Schuffner s dots are often regarded as characteristic of *P vivax* infections but similar stippling occurs freely in *P ovale* infections especially during the stage in which the plasmodium is half-developed becoming less obvious in the schizont stage

Most observers agree that Schuffner s dots do not occur in quartan malaria but stippling of a somewhat similar kind has been occasionally reported in infections with this plasmodium The evidence for the existence of such changes in the red cells in *P malariae* infections was reviewed by Stephens and Owen (1927) who pointed out that such observations are opposed to the statement of the great majority of observers that the quartan parasite produces practically no changes in the red cells

Stippling of the invaded red cell is often seen in *P falciparum* infections especially in heavily stained blood films Here it takes the form of scattered coarse brick-red dots and streaks usually called Maurer s dots The relation of these to Schuffner s dots has not been determined They are usually much less numerous than are Schuffner s dots

The stippling of red cells described above has not been satisfactorily explained Schuffner himself thought that the dots were probably part of the essential structure of the invaded erythrocyte which became susceptible to staining only after invasion by the parasite Sinton and Ghosh (1934) concluded they were local aggregations of haemoglobin derivatives Meyer and Rieder (1907) however had suggested that Schuffner s dots might in fact be related to the reticulum of the young red cell and Hungst (1936) after a study of induced *P vivax* malaria concluded that this was indeed the case He found that in two cases of *P vivax* malaria 5 5 and 6 4 per cent respectively of the invaded cells contained reticular material Schuffner s dots were present in 3 8 and 8 6 per cent of the cells containing rings so that the total percentage

of parasitized cells containing either reticular material or Schuffner's dots was high Hungst considered that it was reasonable to suppose that the cells showing Schuffner's dots were probably in the reticulocyte stage when invaded and concluded that his observations supported the view that the dots were reticular in origin Malamos (1937) could however find no evidence to support this hypothesis He found the staining reactions of Schuffner's dots completely distinct from those of reticulum and in his *P vivax* cases noted a high percentage of cells with associated Schuffner's dots although the degree of invasion of reticulocytes was in his cases very small Further work is required before it can be finally decided whether or not Schuffner's dots are remnants of cell reticulum The determination of the porphyrin content of invaded cells might help to settle the matter

CHANGES IN RED CELL PIGMENT

When the merozoite first invades the red cell it is unpigmented but as the plasmodium develops within the intact erythrocyte granules of brownish or black pigment appear the features of which depend upon the species of invading plasmodium and its rate of growth As the parasite develops a change can frequently be observed in the colour of the host's cell which becomes paler than the non infected erythrocyte This loss of colour is usually well seen in *P vivax* (Stitt) and *P ovale* (Stephens 19—) and is presumably related to the disappearance of haemoglobin following its utilization by the parasite A reduction of the haemoglobin concentration in the peripheral blood in malaria infections has of course been noted in all forms of human malaria and has been shown to run roughly parallel with the reduction in numbers of red cells The loss of intracellular haemoglobin in the invaded erythrocytes has not been actually shown in human malaria but Christophers and Fulton (1938) have demonstrated it indirectly in *P knowlesi* infections in monkeys

HAEMOLYSIS

Phagocytosis

The destruction of invaded red cells has been referred to above Such loss of red cells takes place partly as a direct result of the growth of the parasite and the destruction of the host's cell at the time of sporulation and partly through phagocytosis of both invaded and uninvaded erythrocytes The phagocytosis of red cells has been observed by many

authors during malaria both in the peripheral blood and in the internal organs particularly the spleen and bone marrow Taliaferro and his associates have shown that phagocytosis is carried out mainly by the cells of what they call the macrophage-lymphoid system which includes the reticulo-endothelial cells of other authors These cells phagocytose parasitized and unparasitized cells alike In monkey malaria such phagocytosis is especially prominent in acute infections at the so-called crisis of the infection According to Taliaferro the heightened phagocytosis at this stage of the disease indicates the appearance of acquired immunity and may possibly be explained by the development of opsonins (Coggeshall 1937) It is important here to realize that phagocytosis of red cells although an important factor in erythrocyte loss in malaria seldom proceeds at a rate sufficient to account for more than a small proportion of the loss of red cells from the circulation Phagocytosis of parasites and red cells is not always obvious in malaria infections particularly in human malaria and in severe infections where red cell loss becomes acute the phenomenon may be regarded as inconsiderable The haemolysis occurring in severe malaria must therefore be regarded as intravascular in origin The striking effects of such rapid haemolysis in malaria are best seen in those cases in which the destruction is so rapid that free pigment is thrown out in the urine e g in blackwater fever and the haemoglobinuric fevers in monkeys

Mechanism of haemolysis

The mechanism of haemolysis in malaria has not yet been determined The existence of specifically haemolytic parasite strains has been postulated but not demonstrated (Giglioli 193-) No circulating haemolysin has been identified either in malaria or in blackwater fever although there is some evidence that the exciting agent is extracellular Foy and Kondi (1943) found that red cells from normal donors were rapidly haemolysed when introduced into an actively haemolysing case of blackwater fever and inferred from this that in blackwater fever some circulating haemolysin may exist Foy and his colleagues however discovered that cells from a haemolysing blackwater fever case were equally rapidly lysed when introduced into a *normal* circulation They suggested that the extracellular agent responsible for the lysis of normal cells in the blackwater fever circulation might also be capable of rendering the red cells abnormally susceptible to destruction by the normal lytic processes

Changes in the red cells themselves have been considered by some

authors to be responsible for their increased susceptibility to lysis. The changes occurring as a result of direct invasion of the cells can, as we have said, be of little importance in regard to the total degree of anaemia produced except in extreme degrees of parasitaemia such as are met in *P. knowlesi* infections. Attention has therefore been concentrated more on the mode of destruction of the non-parasitized erythrocytes. Alterations in saline fragility have been recorded by some authors, but on the whole a decreased rather than increased fragility has been observed: thus Barrenscheen and Glaessner (1923) and Potapenko (1929) observed some increased fragility, whereas Dudgeon (1920) and Kligler both observed decreased fragility and Garin (1930) and Foy and his colleagues (1943) observed no change.

Ross (1928) investigated the saline fragility of erythrocytes in ten cases of *P. falciparum* malaria and six cases of blackwater fever using Simmel's technique (1923). He found no appreciable change from normal in the cells in either condition. He considered that the haemolytic processes in the blackwater fever must therefore be independent of the saline fragility of the erythrocytes. He noted, however, that Barcroft had reported that red cells from the splenic pulp were more fragile than those in the peripheral blood, and suggested that if haemolysis occurred mainly in the internal organs such as the spleen, in which the infected cells became filtered off from the circulation, measurements of the fragility of the red cells in the peripheral blood were of no real value, whereas if the haemolysis went on in the blood stream, which was more likely, the results showed that osmotic processes were not involved in it.

Garin (1930) found no difference between the saline fragility of invaded and non-invaded erythrocytes.

Foy *et al.* have recently reported that the red cells during the lytic phase of blackwater fever may show an increased fragility to the action of a lysolecithin system, although at the time the saline fragility is undisturbed. The significance of this finding is not altogether clear, though it is probably associated with the observations of Foy and his colleagues and others that in malaria and blackwater fever some degree of spherocytosis may be found. According to some workers, e.g. Yorke, Murgatroyd and Owen (1930) and Fairley *et al.* (1938), the mean cellular volume is not changed in blackwater fever or chronic malaria, but spherocytosis has been described directly or indirectly by several authors including Gear (1946), who states that in two cases of malaria the red cells were microcytic and hyperchromic, which in his view showed a tendency to spherocytosis. Foy and Kondi (1943)

measured the mean corpuscular volume in their case and found it considerably increased and the diameter/thickness ratio of the cell reduced The appearance of spherocytosis suggests that the increase in lysolecithin fragility is related to the action of this substance on the lipoid and lipoprotein complex in the cell membrane (Foy and Kondi 1943) allowing alteration in the cell volume and possibly concomitant changes in the permeability of the membrane to haemoglobin

The development of spherocytosis may be related directly to the ultimate destruction of the cell since Whipple (1941) suggests that spherocytes may obstruct the circulation through the small blood vessels and so retard the passage of cells through the internal organs particularly the spleen thus encouraging phagocytosis In this connection it should be noted that the slowing of the circulation itself may aggravate the degree of spherocytosis of the red cells concerned and that this also assists the process of lysis (Ham and Castle 1940)

Fahraeus and his colleagues have shown that when defibrinated blood is allowed to sediment at body temperature and is subsequently shaken up again sedimentation after the shaking is slowed and the red cells become more globular than normal This process they call stabilization and they consider it is due to the appearance at the time of separation of corpuscles and plasma of a chemical substance allied to if not identical with lysolecithin According to these workers separation of the blood elements takes place in the spleen (equivalent to allowing warmed blood to settle *in vitro*) and stabilization occurs when the cells and plasma meet again in the splenic vein (equivalent to shaking) In support of this suggestion many authors have observed that blood from the splenic vein has a much slower sedimentation rate than blood from the splenic artery and that cells from the splenic vein are more spheroid than those from the artery The stabilizing substance is normally adequately adsorbed on to the surface of erythrocytes but when the erythrocyte surface is reduced e g in anaemia adsorption is incomplete this results in lowering of sedimentation rate together with the appearance of more globular erythrocytes which develop ultimately into spherocytes According to Vint (1941) the slowing of the circulation through the spleen which occurs in malaria particularly if it goes on to the stage of stasis will facilitate the separation in that organ of the erythrocytes and plasma This increased separation and the prevailing malarial anaemia would allow an increased degree of stabilization in the splenic vein and possibly account for the spherocytosis and so lead to increased haemolysis

(*Bergenhem and Fahraeus 1936 Stephens 1939 a and b 1940* Fahraeu 1939 Vint 1941 Knisely 1934)

Other changes in the red cell particularly changes in its surface *may affect its chances of destruction The possibility of stimulation* of phagocytosis by the production of opsonin has already been mentioned but certain other changes concerned with the phenomenon of phagocytosis *may be mentioned here Brown (1933)* has demonstrated a reduction in the electrical charge on the surface of the erythrocytes (both parasitized and unparasitized) in bird malaria and suggested *that as in the case of bacteria this might be related to the* development of immune substances in the blood and be partly responsible for the increased phagocytosis of red cells in malaria Variations in the *surface charges of the erythrocytes have not yet been reported* in human malaria but the changes of sedimentation rate which have been demonstrated could be explained on this basis Investigation of the erythrocyte *surface phenomena in malaria might* also help to interpret recent observations of Knisely in regard to the agglutination of red cells in monkey and human malaria Knisely (1945) showed by direct observation of the circulation in severe malaria that at a certain stage of the infection a substance which he identified as fibrin appeared as a fine precipitate around the erythrocytes The appearance of this substance was followed by clumping of the erythrocytes into what he described as a sludge which interfered with the passage of blood through the finer blood vessels Knisely also noted that red cells coated with the precipitated fibrin became sticky to macrophages the appearance of the precipitate coinciding with avid phagocytosis of both parasitized and unparasitized cells This phenomenon will be discussed in more detail later

The changes recorded in the erythrocyte sedimentation rate in malaria also indicate some alteration in the surface charge and may be associated with the autoagglutination of red cells so commonly seen in severe malarial anaemias It has been suggested by some authors that autoagglutination is in many cases a preliminary stage to lysis

It will be seen that changes in the red cell cannot alone account for the haemolysis which occurs in malaria and it is generally agreed that associated changes in the external environment of the red cell must co-exist with the changes in the red cells themselves Ponder (1944) considers that in all probability the normal intravascular lysins of the body play little part in red cell destruction unless their concentration rises or unless their activity is increased by acceleration or by reduction of the activity or concentration of the normal inhibitory

mechanisms He believes that the three normal *in vivo* lytic processes are (i) the action of bile salts and soaps (ii) the action of the spleen in giving rise to substances leading to spherocytosis of the red cells identified by Bergenhem and Fahraeus as lysolecithin (small amounts of which produce spherocytosis large amounts haemolysis) and (iii) haemolytic substances derived from the tissues Lytic processes such as saponin haemolysis are inhibited by various substances present in normal plasma including cholesterol and the plasma proteins especially globulin Under certain circumstances the inhibitory action of these substances may be further enhanced or diminished with consequent changes in lysis The inhibitory power of human plasma according to Ponder is thus related to the combined concentrations of cholesterol protein and possibly lecithin rather than to the concentration of cholesterol alone Changes in the concentration of these substances have been frequently reported in malaria and may have some direct effect on the normal haemolytic processes of the body leading under special circumstances to excessive activity Maegraith Martin and Findlay (1943) reported that animal tissue contains a heat labile lytic agent which can be inhibited by various means such as heat and by substances present in tissue washings and serum They have suggested that the rate of lysis occurring in the normal animal is dependent so far as these tissue lysins are concerned upon the balance between the lytic agent and its inhibitors Evidence obtained from blackwater fever indicated that this balance might be shifted to the lytic side in that disease so that the haemolysis observed might be in fact simply a manifestation of excessive uninhibited activity of a normal lytic process Ponder (1944) has repeated the experiments of Maegraith and his co-workers and confirmed their observations as far as the lytic effects of various tissues are concerned Weil (1907) found similar evidence of the presence of lytic agents in the tissues and observed that the haemolytic activity of the tissue agent is enhanced by the addition of extracts of red cells (his RBCD factor) He noted that the haemolytic agent present in organ extracts could be anchored to the red cells and that cells thus sensitized could be haemolysed by the addition of the RBCD factor Weil s demonstration of this red cell factor may be of importance in malaria in that the products of red cell destruction may assist haemolysis once it has started Bruckmann and Wertheimer (1945) confirmed the presence of the lytic agent in tissue but considered that it was not connected with either normal or pathological blood destruction Evidence has also been provided by Brown *et al* (1944) of the existence of a tissue lytic factor during the course of

experiments designed to investigate the fate of transfused red cells in anaemias of various kinds Ponder suggested that the tissue lytic agent described by Maegraith *et al* may be lysolecithin and so related to the stabilizing factor of Fahraeus In this case it is probably not the same substance as that recently isolated by Laser and Friedmann (1945) from human plasma This latter substance is nitrogen and phosphorus free and cannot therefore be lysolecithin Its significance in malaria has not been established but the remarkable inhibition of its activity by antimalarial drugs has led Laser to suggest that the malaria parasite may produce during its development a metabolite closely related to this haemolytic agent

On the analogy of the part played by the spleen in other lytic anaemias a splenic factor has frequently been postulated as an agent in the process of haemolysis in malaria Reference has already been made above to the production of the stabilizating factor in the spleen following separation of plasma and red cells during the passage of blood through the organ Fahraeus thought that this factor was probably related to lysolecithin and was an important agent in haemolysis Vint (1941) has pointed out that the enlargement of the spleen which occurs in malaria and blackwater fever would tend to increase the degree of separation of erythrocytes and plasma in that organ and so influence the production of the stabilizing factor Foy and Kondi and Gear (1946) have also discussed this possibility The latter has further suggested that in blackwater fever the spleen may act as a reservoir of autohaemolysin which may be forced into the general circulation during splenic contraction and account for the sudden onset of haemolysis seen in blackwater fever The production of excess of the stabilizing factor in the enlarged spleen of malaria has not yet been demonstrated but the theoretical possibility exists particularly in view of Knisely s observations on splenic blood flow It should however be pointed out here that the latter s observations have not yet been fully confirmed by other investigators

Variation in the physico-chemical environment of the erythrocyte may play an important part in haemolysis Thus changes in the reaction of the plasma in the acid direction have been shown to be associated with increased red cell destruction in certain haemolytic anaemias For instance Dacie and Richardson (1943) have shown that in patients suffering from haemolysis associated with nocturnal haemoglobinuria lysis of the red cells is sensitive to pH changes both *in vivo* and *in vitro* They found that in *in vitro* haemolysis the optimum for lysis occurred at pH 7 _ and inhibition of lysis about pH 8 and pH 6

Birnbaum Goldblum and Kligler (1946) observed that red cells from malaria patients laked more easily in bile solution than did cells from normal subjects Quinine enhanced this lytic effect and alkali inhibited it They considered that the inhibitory effect of alkali was not primarily due to pH changes and that though acidity might play some part in haemolysis it is probable that it is the red cell itself which is chiefly concerned its sensitivity to lysis is therefore not primarily related to its external environment Smith and Evans (1943) however claim that alterations to the blood pH may affect the rate of haemolysis These authors found *in vitro* as have many others that erythrocytes were more resistant in alkaline than in acid solutions of saline They suggested that the degree of acidosis sometimes seen in blackwater fever may account for the excessive haemolysis seen in that disease The acidosis in malaria and blackwater fever however when it does occur is not to be measured in terms of gross deviation of the pH of the blood At most it is exhibited by small changes in alkali reserve and cannot seriously be considered important so far as haemolysis is concerned The fallacy upon which the claim of Smith and Evans is based may be stressed by the work of Harkins and Hastings (1931) which illustrates the extremes of acidity necessary to disturb the buffering effect of the plasma sufficiently to alter the pH *in vivo* for any length of time

It is possible that haemolysis in malaria may be the outcome of some form of immunity reaction involving antigen-antibody complexes and possibly complement The evidence for such reaction in malaria and particularly in blackwater fever has recently been reviewed by Gear (1946) He claims that the presence of antibodies in the form of opsonins is indicated by the increased erythrophagocytosis demonstrated during malaria in the organs of the body and the peripheral blood (Taliaferro and Mulligan 1937 Yorke Murgatroyd and Owen 1930 Thomson 1924) Coggeshall (1937) supports this view The existence of complement deviating substances in the serum of cases of human and monkey malaria also clearly demonstrates the presence of antigenic complexes within the invading parasites The development of spherocytosis as a possible preliminary to haemolysis is according to Gear suggestive of the existence of autohaemolysins and Foy s experiments on the fate of transfused cells in blackwater fever and normal circulations can also be explained on the basis of sensitization of the red cells concerned Gear points out that the paroxysmal haemoglobinuria and incompatible blood transfusion may both be regarded as examples of haemolysis associated with antibody

reactions He also draws attention to the many similarities between the syndrome of blackwater fever and the effects of haemolytic sera Gear has propounded a theory of haemolysis which is of considerable interest He found in unpublished experiments that an emulsion of liver from a rhesus monkey killed by yellow fever produced liver antibodies after injection into other rhesus monkeys protected against yellow fever The production of such homologous antibodies for liver was similar in many ways to the results of the experiments of Schwentker and Comploier (1939) who observed the production of complement fixing antibodies reacting with rabbit kidney and brain following injection into rabbits of emulsions of homologous kidney plus staphylococcal and streptococcal extracts On the strength of these observations Gear has suggested that the lysis of red cells in blackwater fever (and presumably in malaria) may arise from the activity of an autolysin produced as follows

(i) Red cell + malaria parasite
or + + antimalarial drug = autoantigen
or + antimalarial drug

(ii) Autoantigen + reticuloendothelial system = autoantibody (haemolysin)

(iii) Red cell + haemolysin = sensitized red cell

(iv) Sensitized red cell + complement = lysis

He concludes that red cells invaded by malaria parasites become autoantigenic An antibody (or haemolysin) to red cells is formed in the reticuloendothelial system particularly in the spleen When the splenic circulation is unobstructed this haemolysin is mopped up by red cells and these sensitized cells are removed by the reticuloendothelial cells as they are sensitized and before demonstrable intravascular haemolysis occurs When the spleen becomes congested as it does in malaria the impedance of the circulation allows the haemolysin to accumulate and under certain conditions this accumulated haemolysin may be thrown in large amounts into the general circulation Gear considered that the exhibition of antimalarial drugs may affect the production of the autoantigen

In many ways Gear s hypothesis helps to explain the phenomenon of malarial haemolysis but at the present stage its chief virtue lies in the fact that it offers a new basis for experimental work

The possibility that immune reactions may be concerned in the haemolysis of malaria is further suggested by the extraordinary degree of agglutination (which some regard as a preliminary step to haemo-

lysis) which is frequently seen in severe malaria This phenomenon is sometimes easily visible microscopically especially when the red cell count is of the order of two million or fewer per cu mm Oliver-González has suggested that the malarial plasmodium may contain an antigen related to human isoagglutinins and that the autoagglutination seen in blackwater fever and malaria may result from immunization of the host against this substance An increase in alpha and beta agglutinins has also been observed in cases of malaria and blackwater fever (Oliver-González 1944) and may be associated in some way with the excessive agglutinability of the red cells in these diseases In any case such changes in the agglutinin pattern of the plasma may explain the extreme difficulty sometimes experienced in finding suitable blood donors for transfusion of malaria patients Cold agglutinins have been found by several workers in the sera of patients suffering from malaria and blackwater fever and may possibly be connected with the phenomenon of autoagglutination as they are in certain other diseases Butts (1945) believes that a factor concerned in the development of blackwater fever haemolysis may be isoimmunization to an Rh-like substance present in the malaria plasmodium He supports his suggestion by quoting figures for the incidence of blackwater fever and erythroblastosis foetalis in whites and negroes

Enough has been said above to show that there are grounds for believing that immunity reactions may be important in the production of haemolysis in malaria but at present too much stress should not be laid on this proposition particularly in regard to intravascular agglutination which Knisely has shown may also occur in some degree as a result of crush trauma

At least two other possible mechanisms may be concerned in haemolysis in malaria namely the liberation of malarial pigment at the completion of schizogony and the production of poisonous substances by the parasite either directly (toxins) or indirectly in the form of metabolites such as the products of incomplete carbohydrate oxidation e g pyruvic or lactic acids The careful work of Morrison and Anderson and others on the part played by malarial pigment in the pathogenesis of the disease leaves little doubt that the pigment as such is of no importance in the production of lysis As far as the products of metabolism of the parasite are concerned or the production of a toxin by the plasmodium the evidence is meagre and conflicting and will be discussed elsewhere

SEDIMENTATION RATE

Changes in erythrocyte sedimentation rate in malaria have been described by many authors Occasionally a reduction in this rate has been reported but more frequently an increase had been observed particularly during the acute stage of the disease Radosavljevic and Ristic (1936) demonstrated such an increase in sedimentation rate and a return to normal subsequent to the cure of the acute attack The sedimentation rate in three forms of monkey malaria and in human malaria was investigated by Kehar and Harbhagwam (1937) Observations were made in five monkeys before inoculation with *P knowlesi* and subsequently during the incubation period and active disease In the incubation period the sedimentation rates were unaffected but during the active disease a notable and progressive increase in rate occurred particularly in the last stages of the attack when there was intense parasitaemia and a marked fall in erythrocyte count There was a clear relation between the total red cell count and the rate of sedimentation (and therefore associated parasitaemia) In animals given adequate antimalarial treatment sedimentation rates fell rapidly to normal limits In chronic infections with *P cynomolgi* and *P inui* the sedimentation rate was slightly increased In relapses there was a sharp rise

In human *P vivax* *P falciparum* and mixed infections also an increase in sedimentation rate was observed (Mulligan) in some cases the rate was over 60 mm in 1 hour Sedimentation in *P falciparum* was not apparently greater than in *P vivax* infections and there was no obvious correlation between the degree of sedimentation and parasitaemia body temperature or size of spleen

Kehar and Harbhagwam noted an almost proportional increase in sedimentation rate with the rise of the globulin fraction in the plasma of monkeys in acute *P knowlesi* infections The surface tension of the blood was also lowered in the acute final stages of the disease Wood (1945) has confirmed the increase in sedimentation rate in both *P vivax* and *P falciparum* malaria There was no obvious difference in the sedimentation rate observed in these two infections or between the rates noted in acute attacks primary attacks or relapses The changes in sedimentation rate lasted from two to three weeks after the acute attack

The increased sedimentation rate observed in malaria probably depends partly upon alterations in the cells themselves such as changes in the surface electrical charge and in the shape of the corpuscles

(Stephens 1940) Changes in the physico-chemical constitution of the plasma are probably also concerned such as quantitative alterations in plasma protein as suggested by Kehar and Harbhagwam and possibly to a minor degree changes in the ionic concentration of salts In human infections in contrast to monkey malaria no close relation has been observed between the degree of anaemia and the rate of sedimentation

COAGULATION TIME

Bleeding time in malaria appears to be little altered (Mikeladse 1924) Reduction of the coagulation time during the paroxysm has however been recorded by Maslova (1924) The changes in fibrinogen are sometimes equivocal but a decrease in this globulin has been noted by Kopp and Solomon (1943) in induced benign tertian malaria Radosavljevic and Ristić (1926) claim however that there is sometimes an increase in fibrinogen during the paroxysm at the height of fever No significant changes in prothrombin values have been recorded (Fredricks and Hoffbauer 1945 Diggs 1945) Decrease in platelets has been reported during the paroxysm (Maslova 1924)

LEUCOCYTES AND PHAGOCYTES

The total leucocyte count in human malaria may sometimes rise above normal in the early stages of the acute attack especially in *P falciparum* infections (James 1922 Garin 1930) The leucocytosis may persist in pernicious forms of malaria particularly when the temperature is rising (Marchiafava and Bignami 1900 Craig 1909 James 1922) Most authors however are agreed that during the ordinary malarial attack a mild leucopenia develops the white count often commencing to fall before the appearance of parasites in the peripheral blood Fairley and Dew (1920) for instance reported a constant leucopenia in both pyrexial and apyrexial naturally acquired *P falciparum* infections associated with a granulocytopenia and an increase in mononuclear and transitional cells A leucopenia has also been reported by Fairley (1947) in induced *P falciparum* and *P vivax* infections Kitchen (1941) in ten cases of *P falciparum* infection found that the leucocyte count dropped from an average of 7 5 to about 6 0 thousand cells per cu mm in the three days immediately preceding the appearance of parasites in the blood Thereafter it fell slowly, reaching a minimum of 5 0 thousand cells per cu mm on the sixth day Treatment or spontaneous cure lead to a return of the leucocyte to normal

values although in some cases the leucopenia persisted for some time In chronic malaria there is usually some degree of leucopenia and in relapses there may be a greater reduction in leucocytes than that observed in the primary attack

Fitchen considers that the leucopenia of malaria is probably non-specific in origin and is of the same general type as that initiated by the administration of foreign protein

Characteristic findings are those recorded by Bianchi (1940) in acute *P falciparum* infections In 12 cases the total white cell count varied from 4 0 to 6 0 thousand cells per cu mm. The lowering of the leucocyte count was primarily due to a fall in the numbers of neutrophil cells Total numbers of lymphocytes were slightly increased and there was a clear increase in monocytes The average differential count in the 12 cases was neutrophils 50 per cent lymphocytes 34 per cent monocytes 12 per cent Similar figures have been reported by other workers A fall in neutrophils and a rise in monocytes has been observed also by James (1922) and others

A rise in neutrophil numbers has been observed by some authors in *P vivax* malaria Garin describes such a rise taking place just before the beginning of the paroxysm succeeded by a reduction as the fever subsided In spite of such fluctuations there was a gradual decrease in neutrophils and a shift to the left as the disease progressed associated with a fall in total white cell count Treatment or spontaneous cure was followed by a gradual return to normal of both total leucocyte and neutrophil count Garin also noted a relative mononucleosis and some eosinophilia most marked in the apyrexial intervals

Schilling (1924) also reported an increase in numbers of neutrophils in the early stages of the paroxysm associated with a progressive decrease in monocytes and lymphocytes He observed a decided shift to the left in the granulocytic cells at the beginning of the paroxysm An increase in total leucocyte numbers during the early stages of fever was recorded by Rubitschung (1925) who stated that as the disease progressed the curve of leucocyte numbers corresponded approximately to the inverted fever curve There was also an inverse relation between the numbers of parasites and leucocytes such as is seen in the terminal stages of malarial infections in monkeys

The changes in the leucocyte count in malaria have been carefully followed by Malamos (1934) in *P knowlesi* infections in *M rhesus* *M mulatta* and *Cercopithecus mona* The leucocyte count in normal animals ranged from 15 600 to 24 000 per cu mm Shortly after experimental inoculation of the malaria a mild leucocytosis developed

This was followed by a leucopenia and a return to normal values after treatment The leucopenia was as in human malaria primarily due to a reduction in the numbers of neutrophils A shift to the left was not observed in the acute clinical stage of the infections but appeared later in the regenerative phase when the bone marrow was restoring the normal balance of cells Lymphocyte numbers were not altered appreciably There was an increase in monocytes from the beginning of the infection In splenectomized animals which died rapidly from the infection there was a terminal fall in numbers of all leucocytes including lymphocytes and monocytes The findings of Malamos are similar to those of Krishnan Lal and Napier (1932) who also noted a leucocytosis at the beginning of the first stage of the acute disease in monkeys These authors studied the development of monocytosis and found that on the first day of the appearance of parasitaemia there was usually already a rise above normal (6 8 per cent normal 4 4 per cent) On the second day the monocytes rose to 11 5 per cent When parasitaemia was extreme the monocyte count fell (7 5 per cent) After treatment with quinine or spontaneous recovery there was a further transient rise of monocytes

Kehar (1936) also found a leucocytosis in the early stages of the acute disease in *P knowlesi* infections in *M mulatta* In three monkeys the normal leucocyte count varied from 13 8 to 18 0 thousand cells per cu mm A rise was observed in all three on the day preceding the appearance of parasites in the blood and thereafter the count rose to between 24 and 32 thousand per cu mm until the day of death in one case and the final two days in the others when there was a considerable fall (between 17 and 25 thousand per cu mm) corresponding to an enormous increase in parasitaemia

Stephens (1937) reviewed the literature pertaining to leucocyte changes in blackwater fever and stated that there was much discrepancy between the values observed by various authors for absolute and relative cell counts Both leucocytosis and leucopenia had been recorded and sometimes both at different times in the same patient An increase in monocytes had been noted by many authors but not by all For instance Stephens and Christophers (1901) recorded monocyte counts as high as 23 per cent whereas Fairley and Bromfield (1933) reported a *non-fatal case in which over a period of 19 days* the number of monocytes never exceeded 4 per cent of the circulatory leucocytes A reduction in true lymphocytes was noted in some cases by Christophers and Bentley (1908)

Yorke Murgatroyd and Owen (1930) described a case of blackwater

fever in which haemoglobinuria persisted for the first four days and in which there was a leucopenia for the first three days a normal count on the fourth day and a decided leucocytosis on the fifth and seventh days of the illness (18 . and 12 5 thousand cells per cu mm respectively) They drew attention to the experimental work of Mackenzie (1929) and Uchida (19.1) on the changes in the white cells following artificially induced attacks of paroxysmal haemoglobinuria both of whom observed leucocytosis following haemolytic paroxysms Uchida found similar leucocyte changes in rabbits after injection of blood derivatives and believed that the blood changes in patients were due to the products of destruction of the erythrocytes Such patients according to this worker produced a phagocytosis promoting substance (auto haemotropin) in the blood Yorke and his colleagues suggested that the leucocytosis observed in their case may have represented an attempt on the part of the organism to deal with the stroma of haemolysed erythrocytes

A similar process may be going on in malaria uncomplicated by haemoglobinuria It was considered at one time that malarial pigment might be responsible for the changes in leucocytes since Brown (1913) found that in the rabbit intravenous injection of alkaline haematin gave rise to immediate leucocytosis (such as Garin reports in man and Malamos in monkeys) and a rapid increase in monocytes and pigmented phagocytes in the peripheral blood The role of parasitic pigment as a specific factor has however recently been denied since in the disease the pigment is apparently never liberated into the blood stream in soluble form (Anderson and Morrison 1942)

Evidence of phagocytosis is commonly seen in the cells of the blood in acute malaria Thomson (1933) states that after a paroxysm the appearance of pigmented monocytes is comparatively common Most observers have at some time or other reported pigmented leucocytes in the peripheral blood but as Taliaferro and Mulligan have shown these cells are usually monocytes and rarely polymorphs The latter however do occasionally contain parasitic pigment but the number of such pigmented cells is usually small and the content of pigment low Polymorphs as a rule contain only pigment but in very acute or pernicious *P falciparum* infections they may show ingested parasites as well Thomson (1933) refers to three cases of acute *P falciparum* malaria in which the circulating polymorphs contained both pigment and parasites the latter in the late schizont stage The latter stained well and showed no signs of degeneration Similar phagocy-

tosis of parasites by polymorphs in the peripheral blood has also been recorded by Thomson and Robertson (1929) and de Langen (1933)

In monkey malaria pigmented polymorphs are rarely seen in the peripheral blood Taliaferro and Mulligan (1937) have repeatedly failed to find much evidence of parasite phagocytosis by polymorphs (heterophils) in even the most severe malaria infections in monkeys associated with very high parasitaemia They cast some doubt (almost certainly unjustified) on the validity of the evidence of Thomson and others in regard to phagocytosis of parasites by polymorphs and suggest that some of the cells described were not polymorphs at all

CHAPTER IV

THE BLOOD IN MALARIA AND BLACKWATER FEVER

PLASMA PROTEINS: Albumin and the globulins. BLOOD SUGAR. CHOLESTEROL AND PHOSPHOLIPOIDS. INORGANIC CONSTITUENTS: Plasma Potassium and Sodium — Chlorides — Others. ALKALI RESERVE. OTHER CHANGES IN THE BLOOD. BLOOD PIGMENTS.

PLASMA PROTEINS

Albumin and the globulins

THE concentrations of the plasma proteins have been found to alter quantitatively in both naturally acquired and artificially induced human malaria and in monkey malaria. Some workers claim that there are also qualitative changes in the globulin fractions, but this has not been substantiated (Trensz 1935; Benhamou and Gille 1935). During the active stages of the disease the total protein tends to fall and there is an associated decrease in albumin and increase in the globulins. Petersen (1926) found a reduction in total plasma proteins during the early stages of the benign tertian paroxysm, the concentration becoming normal again after the paroxysm was finished. A diminution in the concentration of albumin associated with a less obvious fall in total protein concentration at the height of the paroxysm of benign tertian was observed by Radosavljevic and Ristic. Normal values were approached in the apyrexial periods and established rapidly subsequent to treatment. An increase in globulin concentration was observed by Weichmann and Horster during the incubation period in cases of induced malaria and a corresponding fall in albumin which became more pronounced at the onset of the fever. Treatment of the malaria was followed rapidly by restitution of normal concentrations. Lloyd and Paul examined the protein changes in the blood of eight cases of chronic *P. falciparum* and *P. vivax* infections (four of each) over long periods during and after treatment with quinine. They found in all cases before treatment that the total protein was low, the reduction being mainly due to lowering of the albumin concentration. Total globulin was also lowered, but the concomitant fractions were altered so that the euglobulin was roughly doubled and the pseudoglobulin reduced. The globulin albumin ratio was correspondingly altered. They concluded that the fall of protein content of the plasma was the result of

the plasmodial invasion and not directly connected with the existing fever A rapid return to normal concentrations followed quinine therapy the albumin was the slowest to be restored

Ghosh and Sinton (1935) reviewed the literature up to that period and investigated the protein changes in monkey malaria They considered that since a good deal of the information in human cases had been obtained in malaria induced in syphilitics it was necessary to investigate the problem under more controlled conditions They examined the changes in blood protein in infections of *P knowlesi* *P cynomolgi* and *P inui* in rhesus monkeys In blood inoculated severe *P knowlesi* infections during the acute stages of the disease they observed a marked fall in albumin concentration and a slight rise in globulin associated with a fall in the total protein values Their results can best be judged by quoting an example from their experimental series Thus in monkey 556 between the first and the ninth days of the disease the total protein fell from 7 44 to 6 93 gm per cent the albumin from 4 9 to 4 1 and the globulin rose from 2 54 to 2 8 gm per cent In animals suffering from chronic *P knowlesi* infections heterologous or homologous superinfections were followed by similar changes in plasma protein In the intervals between relapses or superinfections the protein values approached normal In *P cynomolgi* infections which were clinically much milder than those caused by *P knowlesi* the changes in the plasma protein were not so evident and in *P inui* infections were inappreciable Treatment of the malaria by various drugs including an arsenical restored normal protein values in both *P knowlesi* and *P cynomolgi* infections The degree of reduction in albumin and increase in globulin depended in *P knowlesi* infections on the severity of the attack and intensity of the plasmodial invasion

Kehar (1936) estimated the serum proteins in three fatal haemoglobinuric cases of *P knowlesi* infection in *M mulatta* recording measurements before inoculation during the incubation period and daily in the active disease up to the day preceding death He found the total protein rose slightly in two monkeys on the day before the clinical signs of the disease appeared and fell in the third animal On the day preceding death the total protein was reduced in the two animals and raised above normal in the third Albumin concentration fell in all three the reduction first showing on the day the parasites appeared in the peripheral blood The globulin fraction rose in all after the onset of the disease reaching its highest values on the day before death Kehar points out that his results very closely agree with

those of Ghosh and Sinton in monkey malaria and Chopra *et al* (1935) in human malaria

Kopp and Solomon (1941) examined the plasma protein changes in neurosyphilitic patients who were given therapeutic malaria and allowed 10 or more paroxysms before quinine treatment They observed a rapid and progressive reduction in total protein and the albumin concentrations during the active clinical phase of the malaria and a rise in globulin starting later usually after the first few paroxysms The rise of globulin was roughly inversely related to the fall in albumin in that the maximal concentrations of the former were evidenced at about the same time as the minimal concentrations of the latter Termination of the malaria with quinine was followed by restoration of normal values within three weeks Kopp (1942) in other experiments on neurosyphilitic patients found that the fall in albumin concentration occurred in all cases and was marked and progressive The concentrations of globulin and fibrinogen however showed no consistent trends Normal values were re-established after treatment Fever induced by typhoid vaccine or inductothermy did not appreciably affect the plasma protein levels In subsequent experiments in collaboration with Solomon Kopp (1943) found that the fibrinogen was generally decreased during the clinically active stages of induced benign tertian malaria returning to normal three to six weeks after the termination of the malaria with quinine (The fibrinogen fell to an average of 303 3 mgm per cent in eight of nine patients the average before therapy being 367 8 mgm per cent)

Similar results with regard to plasma proteins have been reported by Boyd and Proske (1941) in experiments designed to determine the relation between the changes occurring in plasma proteins and the clinical appearance of oedema in induced *P vivax* *P malariae* and *P falciparum* infections They observed an increase in albumin concentration in five cases of *P vivax* infections commencing early in the incubation period and giving place to a considerable fall in concentration with the onset of clinical malaria often to as low as 50 per cent of the normal value In two of three cases of *P falciparum* there was a fall of albumin concentration throughout the incubation period continuing into the fever period in one case to below 2 gm per cent In some cases the depression of plasma protein was still present for days or even weeks after the termination of the malaria with quinine In others the levels were restored to normal before treatment

Three patients developed oedema during or subsequent to malarial therapy Two of these were infected with *P malariae* and of these

one showed a depression of albumin corresponding roughly to the period of active malaria and the other showed a fall for the first few weeks followed by a steady rise to normal values continuing during the active stages of the malaria The third patient who developed oedema was infected with *P falciparum* and in this case the plasma albumin fell to less than 2 gm per cent during the clinical activity of the malaria and returned very slowly to normal after treatment

Boyd and Proske found that changes in the globulin fraction of the plasma were not so consistent In some cases there was a clear *rise during the incubation period of the malaria in others the globulin* remained normal until the onset of the fever or even later and then rose The raised globulin concentrations were sometimes maintained for the duration of the malarial attack sometimes for weeks after its termination In one case of *P falciparum* infection the globulin began to rise about a month after the onset of fever and continued to rise throughout the clinical activity of the malaria until a concentration of over 5 gm per cent was attained The concentration of the euglobulin fraction tended to rise in most cases the increase corresponding with that of the total globulin but there was no very close relation between the concentrations of these two fractions Fibrinogen was not estimated but the amount of fibrin recovered after clotting the plasma with salt and calcium chloride remained within normal limits in all cases in which it was measured

The total protein concentration showed a rise when measured during the incubation period followed by a sharp fall soon after the onset of clinical malaria In those cases in which there was an increase of globulin during the febrile period the total protein tended to rise also towards its normal values

Other workers have recently confirmed the fall in albumin levels in the active stages of the disease the relative rise in globulin with associated changes in the albumin/globulin ratio the restoration of normal levels after treatment with either atebrine or quinine (Glenn *et al* 1946 Dole and Emerson 1945) and the relatively longer time required for the readjustment of the globulin level (Lippincott *et al* 1945) Dole and Emerson working on eight cases of naturally acquired relapsing benign tertian infections in which three paroxysms were allowed *found that the albumin/globulin ratio was depressed although* the plasma total protein was little altered the depression being due to the fall in albumin fraction In a single case of *P falciparum* infection however which remained untreated for three weeks there was a great

reduction in total protein and a corresponding change in albumin/globulin ratio These authors suggest that the estimation of total protein and the albumin/globulin ratio might be useful in estimating the severity of the disease

It has been mentioned above that Lloyd and Paul (19[illegible]9) found that the euglobulin fraction of the plasma globulins was much increased Dole and Emerson (1945) investigated the plasma proteins by electrophoretic methods and found that when an increase of globulin occurred during a malarial attack the increase was chiefly observed in the fibrinogen and gamma globulin fractions The motility of the various protein fractions during the malarial attack was normal They considered therefore that the changes in plasma protein accompanying malarial infections are non-specific and consequently methods of diagnosis based on the protein pattern are not significant especially as the latter returns to normal during the latent stage of the disease between relapses although infection must be presumed to continue during this period Diagnostic methods such as that of Proske and Watson (1939) which depend on quantitative determination of euglobulin in serum are therefore probably unsatisfactory and similarly tests such as that elaborated first by Henry (19[illegible]7) which depend on the presence of excess serum euglobulin (flocculated in water in the presence of melanin) are also unreliable (Chorine and Gillier 1934)

The effective osmotic pressure of the plasma is largely dependent on the concentration of albumin and reduction in the latter below a certain point i e about 3 0 gm 100 c c particularly if associated with a reduction in total protein to 5 gm per cent or less may give rise to fluid loss from the capillaries and local oedema (Best and Taylor 1943) Such oedema is occasionally seen in malaria Boyd and Proske (1941) have attempted to correlate it with the protein concentrations prevailing at the time of its appearance They found that no clinical oedema appeared in their five cases of *P vivax* infections although at one time in one patient the total protein and albumin fell below [illegible] and [illegible] 5 gm per cent respectively at the same time and in four the albumin concentration fell below 2 5 per cent The plasma albumin concentrations fell below 2 5 per cent in two *P falciparum* infections on several occasions without the appearance of oedema In a third *P falciparum* infection a value of below [illegible] 5 per cent was obtained at a time when oedema was present but the albumin concentration rose to over 4 per cent during the persistence of the oedema Two cases of *P malariae* malaria developed oedema In both the albumin concentration was depressed to between 2 and 3 gm per cent during

the oedema There was thus no very close quantitative correlation between the appearance of oedema and the existing albumin levels except in the *P malariae* infections although in the oedematous patients the plasma albumin concentrations tended to fall lower than in the non-oedematous cases and stay depressed for longer periods

The development of oedema in relation to plasma protein concentration in malaria has also been studied by Kopp and Solomon (1941) In six of their neurosyphilitics who received *P vivax* infections the plasma albumin concentration fell to less than 3 1 gm per cent The authors calculate that the osmotic pressure of the plasma in these cases must have been reduced to between 16 and 23 mm Hg In spite of this only three patients developed oedema Their results thus confirm the general findings of Boyd and Proske to the effect that protein concentration is not the only factor concerned in the escape of fluid from the capillaries to the tissues They point out that the three patients in whom oedema developed were all anaemic and suggest that this may also have been concerned in the development of the oedema The known effects of severe anaemic anoxia on the endothelium of the smaller vessels make this highly likely (Maurer 1940) particularly as Peters and Eisenman (1933) have shown that other things being equal oedema appears at higher protein concentrations if the subjects are anaemic

√ Plasma albumin and the globulins fibrinogen and prothrombin are synthesized in the liver and variations in either must be related in some degree to functional changes in that organ Albumin is formed slowly and its synthesis is disturbed even in low grade liver dysfunction The fall in concentration in malaria may thus be associated with the hepatic damage which occurs in the disease and has been shown to be accompanied by other signs of functional derangement It may also be reduced as the result of lowered protein intake for long periods before malarial infection e g in poorly fed populations when the body protein stores are already low or following failure of absorption during the acute stages of the disease arising from anorexia vomiting or severe diarrhoea Albumin escapes easily from the plasma through damaged endothelial walls and some may be lost in this way Considerable amounts may be discharged in the urine

In liver dysfunction e g that produced by chloroform or after hepatectomy the concentration of fibrinogen usually falls It is also reduced considerably after haemorrhage Its concentration however varies independently of that of the other protein fractions of the blood under many physiological conditions indicating as Best and

Taylor point out (1943) an independent source It is also important to remember that in mild liver injury where some degree of stimulation as well as depression of the many functions of that organ may occur *the fibrinogen concentration may in fact rise and not fall It is thus* not surprising that although there seems to be a tendency for the concentration to fall in malaria the findings of workers often vary in regard to this globulin No appreciable variations in prothrombin concentration have been recorded during malaria (Fredricks and Hoffbauer 1945 Diggs 1945)

The site of synthesis of the other plasma globulins is not certainly *known but in haemolytic states such as malaria there* is little doubt that haemoglobin from the destroyed erythrocytes serves as the principal source It is also likely that globulins are partly synthesized in the reticuloendothelial system the stimulation of which in the course of malarial infection has been clearly demonstrated in both human and monkey malaria (e g Taliaferro and Mulligan 1937) The fall of albumin and the rise of globulin so frequently recorded in malaria do not appear to be immediately associated since their sites of origin and methods of synthesis are distinct Nevertheless as Himsworth points out (1946) there must be some relation between the two since when the albumin falls from deficiency of protein intake excessive loss from the tissues or deficient synthesis the plasma globulin concentration tends to increase

BLOOD SUGAR

Estimations of the glucose concentration in the blood in cases of human malaria at all stages of the disease have frequently been made over the last 30 years and in spite of the often wide disparity of the results of individual authors *general agreement* has been reached on certain points It is for instance now generally accepted that both increases and decreases in blood sugar may occur in malaria depending on the stage of the disease in which the blood is examined and on the severity or otherwise of the infection The literature was reviewed in 1931 by Sinton and Kehar and more recently by Fulton and Maegraith (1948) Both these reviews have been used in the following summary of experimental results de Langen and Schut (1917) investigated the glucose concentration in the blood of patients suffering from benign tertian malaria in the Netherlands East Indies and observed a rise in concentration which commenced about an hour before the onset of a paroxysm and further rose during the develop-

ment of fever A decrease in blood sugar preceded the fall in temperature at the end of the paroxysm The average glucose content in eight cases during fever was 190 mgm per cent Yoshida and Ko (1920) recorded mean values during pyrexia of 117 125 and 132 mgm per 100 cc for benign tertian malignant tertian and quartan malaria respectively Other authors also observed increased blood glucose during the fever stage but some found little difference from normal and sometimes reported a fall in concentration Massa (19-7) examined the blood glucose concentration of 28 malaria patients and found normal values when there was no fever In primary infections and in relapses occurring after long periods of remission he found that during the rigor the blood sugar rose rapidly and fell to normal at the height of the fever period finally becoming subnormal at the commencement of the apyrexial stage When relapses occurred at short intervals the observed changes in blood sugar were slight Sinton and Kehar state that MacDougall (1930) observed a similar increase during the chill stage of the benign tertian paroxysm in one case and Anderson (1927) reports hyperglycaemia at the beginning of the cold stage falling off as the paroxysm develops At the height of the hot stage the blood sugar sometimes fell to less than the normal fasting concentration On the other hand Ruge (1935) found in a large series of cases including *P vivax* *P malariae* and *P falciparum* infections that estimations made at all stages of the disease revealed that hyperglycaemia was uncommon the glucose concentration never rising above 130 mgm per cent Petersen (19-6) found an actual decrease in blood sugar during the period of chill and rising temperature in inoculated malaria followed by a return to normal concentrations after the finish of the paroxysm Rudolf and Marsh (19-7) and Rudolf (19-7) found that in general paralytics given *P vivax* malaria therapeutically the blood sugar fell as the temperature rose so that in general the glucose concentration appeared to vary inversely as the temperature After paroxysms the blood sugar returned to normal or sometimes became raised above normal fasting values Isolated measurements of blood sugar made for other purposes e g during galactose and laevulose tolerance tests of liver function have revealed equally variable results For instance Sinton and Hughes (1924) and Williams (19-7) found fasting glucose concentrations within normal limits in natural *P falciparum* and induced *P vivax* infections respectively whereas Hughes and Malek (1930) found a mean concentration of 123 mgm per cent in nine cases of chronic malaria Flosi (1944) also reports normal

sugar concentrations in some of his cases of both *P vivax* and *P falciparum* malaria

Sinton and Kehar (1931) investigated the blood sugar changes in 30 white troops in India suffering from chronic infections of *P vivax* and *P falciparum* All patients had parasites in the peripheral blood and had had no antimalarial treatment for some time previous to the experiments which were carried out several hours after the last meal Blood sugar estimations were made soon after the first detection of parasites during fever 12–18 hours after the temperature had become normal and finally after treatment with quinine and pamaquin (lasting three weeks for benign tertian cases and one week for malignant tertian) The blood sugar was measured in all four periods in 25 of the patients The average glucose concentrations found before pyrexia was 66 mgm per cent during fever 124 after fever 75 and after treatment 85 mgm per cent The average value for normal troops in the same area was 85 mgm per cent Very similar figures were obtained by odd sampling at various times in another 38 patients The highest recorded figure was 206 mgm per cent observed in a patient during pyrexia occurring in the course of a *P falciparum* infection

Thus these authors observed in benign tertian a fall in blood sugar prior to the onset of the rigor in agreement with de Langen and Schut (1917) and a rise during the pyrexial period In three cases of *P vivax* infection and one of *P falciparum* the blood sugar concentrations were estimated at frequent intervals over the period of the paroxysm and apyrexial intervals It was found that there was a general tendency for the blood sugar to rise in advance of the temperature and to begin to fall while the temperature was still rising This latter phenomenon was most marked in *P falciparum* infections and probably accounts for some of the apparent disagreement between the observations of various authors although Sinton and Kehar suggest that the findings of Rudolf and Marsh of a pronounced fall in blood sugar during the paroxysm may possibly be due to the syphilitic infections present in their patients

The rise in blood sugar in the pyrexial stages of malaria which has been observed by so many workers has been explained partly in terms of the glycogen content of the liver Thus Sinton and Kehar claim that the degree of rise in a given case depends on the amount of available glycogen in the liver at the time In some very severe cases and in chronic malaria the glycogen may be grossly depleted so that the blood sugar changes would be relatively small In acute fresh infections

ment of fever A decrease in blood sugar preceded the fall in temperature at the end of the paroxysm The average glucose content in eight cases during fever was 190 mgm per cent Yoshida and Ko (1920) recorded mean values during pyrexia of 117 125 and 132 mgm. per 100 cc for benign tertian malignant tertian and quartan malaria respectively Other authors also observed increased blood glucose during the fever stage but some found little difference from normal and sometimes reported a fall in concentration Massa (1927) examined the blood glucose concentration of 28 malaria patients and found normal values when there was no fever In primary infections and in relapses occurring after long periods of remission he found that during the rigor the blood sugar rose rapidly and fell to normal at the height of the fever period finally becoming subnormal at the commencement of the apyrexial stage When relapses occurred at short intervals the observed changes in blood sugar were slight Sinton and Kehar state that MacDougall (1930) observed a similar increase during the chill stage of the benign tertian paroxysm in one case and Anderson (1927) reports hyperglycaemia at the beginning of the cold stage falling off as the paroxysm develops At the height of the hot stage the blood sugar sometimes fell to less than the normal fasting concentration On the other hand Ruge (1935) found in a large series of cases including *P vivax* *P malariae* and *P falciparum* infections that estimations made at all stages of the disease revealed that hyperglycaemia was uncommon the glucose concentration never rising above 130 mgm per cent Petersen (1926) found an actual decrease in blood sugar during the period of chill and rising temperature in inoculated malaria followed by a return to normal concentrations after the finish of the paroxysm Rudolf and Marsh (1927) and Rudolf (1927) found that in general paralytics given *P vivax* malaria therapeutically the blood sugar fell as the temperature rose so that in general the glucose concentration appeared to vary inversely as the temperature After paroxysms the blood sugar returned to normal or sometimes became raised above normal fasting values Isolated measurements of blood sugar made for other purposes e g during galactose and laevulose tolerance tests of liver function have revealed equally variable results For instance Sinton and Hughes (1924) and Williams (1927) found fasting glucose concentrations within normal limits in natural *P falciparum* and induced *P vivax* infections respectively whereas Hughes and Malek (1930) found a mean concentration of 123 mgm per cent in nine cases of chronic malaria Flosi (1944) also reports normal

Kehar the most likely cause of the hyperglycaemia of malaria is the production by the malarial process of increased circulating adrenalin by stimulation either directly or indirectly of the adrenal glands The mechanism of such stimulation they considered might be related to that which gives rise to similar temporary hyperglycaemia in peptone or serum shock or after the injection of histamine (Marriot 1923 Chambers and Thompson 1925) They also draw attention to the similarity between the phenomena of the malarial paroxysm and those of anaphylactic shock and injection of adrenalin (Anderson 1927)

The suggestion made by Sinton and Kehar that the hyperglycaemia of malaria is the result of excessive glycogenolysis in the liver appears to be the most feasible explanation but the way in which such glycogen breakdown can be brought about is not altogether clear If the primary fault is in the liver then the process whereby that organ becomes damaged is probably the same as we have described elsewhere (Chapter VI) namely anoxia and associated changes in blood flow Anoxia resulting from breathing low oxygen tensions was found to give rise in rats to a transient hyperglycaemia followed in fasting rats by a hypoglycaemia hyperglycaemia was absent in adrenalectomized rats which developed an immediate hypoglycaemia The explanation of these phenomena in rats is almost precisely the same as Sinton and Kehar suggest in malaria namely that the first reaction to anoxia is a stimulation of the adrenal medulla and production of excess adrenalin the subsequent changes in blood sugar depending on the supplies of glycogen available (van Middlesworth *et al* 1944) Interference with glycogenesis such as Sinton and Hughes observed in their laevulose tolerance tests in malaria has also frequently been reported in anoxic liver states such as those developing in shock (Saxton and Miller 1944 Clark and Rossiter 1944) It appears highly likely therefore that the elevation in blood sugar seen in malaria can be related like so much else in the disease to a state of induced tissue anoxia

Occasionally as reported above hypoglycaemia has been observed in malaria during the paroxysm. This may to some extent depend on the chronicity or otherwise of the cases examined or on the time during the attack at which the blood sugar was estimated so that the workers concerned e g Rudolf may have missed a transient rise in concentration Sinton and Kehar showed clearly that the hyperglycaemia they observed was of a transient nature and that in some cases the blood sugar fell to normal or below normal values shortly after the height of the paroxysm Fulton (1939) observed hypogly-

on the other hand the hepatic glycogen store would be considerable and the changes correspondingly great

These authors point out that deficiency of liver glycogen might arise in part from defective storage of the sugar taken in the food and quote the results of Sinton and Hughes (1924) and Green (1928) to which might be added those of Williams (1927) in support of the view that in malaria the conversion by the liver of laevulose into glycogen is at fault The recent observations of Lippencott *et al* (1946) are somewhat at variance with these results however since these workers have found that in neurosyphilitics given induced *P vivax* malaria the galactose test which is based on the hepatic conversion of galactose to glycogen was normal Lippencott Ellerbrook *et al* (1945) previously however reported some deviation in this test in chronic relapsing naturally acquired *P vivax* malaria

Sinton and Kehar also suggest that there may be in malaria some defect in the utilization of the blood sugar by the tissues possibly related to the action of insulin In this connection it is interesting to note that Chessa (1938) has reported some degree of sensitivity to insulin in both acute and chronic cases of malignant tertian malaria

Sinton and Kehar discuss at length the possibility that the rise in blood sugar during the paroxysm may be due to excessive breakdown of glycogen in the liver brought about by the increased metabolism of fever damage to the hepatic cells or increased adrenal activity giving rise to an increase in circulating adrenalin

Increased metabolism in fever has been frequently demonstrated For instance du Bois (1922) found that heat production in the chill stage of benign tertian malaria was raised by about 200 per cent Heat loss in the patient in this stage of the paroxysm is greatly inhibited so that body temperature is rapidly elevated Where the temperature rises well above normal in the absence of a high intake of carbohydrate the glycogen of the liver may be broken down to glucose and used for energy supply Thus although Sinton and Kehar state that the rising temperature of the paroxysm does not play any part in glycogenolysis it is possible that some of the glycogen breakdown in malaria is a reaction to the prevailing fever (Kirstein and Bromberg 1939)

Some damage to the hepatic cells has frequently been demonstrated in malaria and it is possible that such injury may give rise at any rate to a temporary hyperglycaemia arising from excessive glycogenolysis such as is seen in chloroform liver poisoning Changes in the physicochemical constitution of the blood (e g changes in pH) reaching the liver may also stimulate glycogenolysis but according to Sinton and

content of the liver The latter however was much lower in the infected animals than in healthy controls (Mean liver glycogen control animals 7 7 infected animals 0 66 (splenectomized) and 0 0_ per cent) He thus demonstrated a diminution of liver glycogen content during acute *P knowlesi* infections but was not able to decide from his experiments whether this was due to loss of storage capacity reduced carbohydrate intake or to demands made on the blood sugar and ultimately the glycogen store by the parasites Marvin and Rigdon consider the latter hypothesis unlikely since the hypoglycaemia observed in ducks infected with *P lophurae* continues even when there is a terminal reduction in parasitaemia These authors suggest however that such a fall in parasitaemia may be related to the hypoglycaemia

BLOOD AND PLASMA CHOLESTEROL AND PHOSPHOLIPOIDS

Most workers have reported a reduction in cholesterol concentration of the blood at some time during the course of a malarial attack Fairley and Bromfield (1933) estimated whole blood total cholesterol concentrations in 12 cases of malaria and found that these were lowered in three of four cases of *P vivax* infection and four of eight *P falciparum* infections (These authors considered values of 120–_00 mgm per cent to be the normal range) Serial investigations were carried out in three cases of induced malaria In all of these after the appearance of parasites the blood cholesterol fell steadily to below normal values rising again upon the termination of the infections by specific drug therapy (quinine in two cases atebrine in one) There was an apparent relation between the recovery of the blood cholesterol after treatment and the development of the reticulocyte response McQuarrie and Stoesser (193_) also found a reduction in blood cholesterol in induced malaria Greig Hendry and van Rooyen (1934) however reported total serum cholesterol concentrations lying within normal limits in several cases in the apyrexial intervals

Crespin and Zaky (1919) found in 30 cases of malaria that the cholesterol content of the blood fell below normal values just before the beginning of a paroxysm and became normal or slightly raised above normal in the pyrexial period and subsequently fell to normal in the apyrexial period In cases in which the temperature remained elevated after the paroxysm the cholesterol tended to fall

Kehar (1937) studied the changes in cholesterol concentration during both *P vivax* and *P falciparum* infections in subjects living in India

caemia in some *P knowlesi* infections in *M mulatta* and found that the lowest blood sugars were present in those monkeys with the lowest glycogen stores in the liver This indicated that as Sinton and Kehar had suggested there was some mobilization of glucose during malaria and that the maximal concentration reached by the blood sugar was related to the glycogen supply available This is also true of blood sugar changes in anoxia, since van Middlesworth showed that the transient hyperglycaemia which resulted from anoxic anoxia in rats was followed by hypoglycaemia in fasting rats but not in well-fed animals in which there was an adequate store of glycogen It is interesting to note that Marvin and Rigdon (1945) have recently reported a severe (60 per cent) fall terminal hypoglycaemia in ducks infected with *P lophurae* They suggest that in such fatal infections in ducks the anoxaemia (or anoxia) resulting from the destruction of the erythrocytes brings about liver damage which impairs liver function so that eventually the maintenance of a normal glycaemia is impossible In birds which were not fasted at the time of the experiment there was a tendency to hyper glycaemia before the parasitaemia reached its peak but in fasting birds there was no appreciable change in blood sugar until the development of the final hypoglycaemia Marvin and Rigdon do not refer to the glycogen content of the livers of their birds Their results however like those of Fulton in monkeys might be interpreted in terms of such concentrations since the body would be unable to maintain normal or raised blood sugar levels once the liver glycogen supply had become significantly diminished These authors showed by injection of insulin that the hypoglycaemic levels reached in the infected ducks were not in themselves sufficiently low to cause death

Christophers and Fulton (1939) found that glucose disappeared rapidly from solution in the presence of *P knowlesi* and Fulton (1939) Maier and Coggeshall (1941) and many others have shown that glucose is an important factor in the metabolism of malarial plasmodia Fulton (1939) has suggested that the plasmodia may act as a continual drain on the glycogen store of the liver He studied this problem in *P knowlesi* infections of *M mulatta* by estimating the glycogen content of the liver and the glucose content of the blood in infected animals at the time of killing He was not able to find any close relationship between the glycogen content of the liver and the glucose concentration in the blood except that in severe infections the lowest values for liver glycogen were found in association with the lowest concentrations of blood sugar The degree of parasitaemia was not closely related to either the blood sugar concentration or the glycogen

were recorded in the same case Fairley and Bromfield reviewed the findings of other workers and reported concentrations of 68–90 and 77–93 mgm per cent respectively in two fatal cases of blackwater fever In three non fatal cases the values ranged from 71–109 mgm per cent Transfusion of blood made little difference to the cholesterol concentration The average for 18 estimations in all five cases was 86 5 mgm per cent There was thus a persistent hypocholesterolaemia

The cholesterol changes in whole blood in monkey malaria were examined by Krishnan Ghosh and Bose (1936) in *P knowlesi* infections in *M mulatta* some of which were splenectomized with a view to increasing the severity of the infection They found the normal values ranged from 143–214 mgm per cent There was no demonstrable change in the blood cholesterol in the incubation period In the early stages of the overt infection great variation in cholesterol values was observed and some reduction occurred in animals in which the parasitaemia developed rapidly where progress of parasitaemia was slow the reduction was not so obvious

After termination of the malaria by quinine the cholesterol concentrations returned to normal or slightly higher values During latent periods in the infection the cholesterol concentrations were just below normal but prior to relapse they rose During a relapse the cholesterol frequently fell lower than in the primary infection and in long-standing cases the authors claimed that there was a state of persistent hypocholesterolaemia

The authors determined the whole blood cholesterol in six infected monkeys with haemoglobinuria associated with severe parasitaemia Serial estimations were made daily from two days prior to the onset of the haemoglobinuria to the day of death In four of these animals there was a decided fall of blood cholesterol concentration 24 hours before the first passage of haemoglobin followed by an equally decided rise within 24 hours of the appearance of the pigment in the urine In all four monkeys the haemoglobin disappeared from the urine although one animal died from the infection Of the other two animals one showed a pronounced fall in blood cholesterol after the onset of haemoglobinuria and died with haemoglobin still present in the urine In neither of these monkeys was there any rise in cholesterol before the onset of haemoglobinuria The other animals which died from severe infections without developing haemoglobinuria showed a big fall of cholesterol concentration within 24 hours of death

Kehar (1937) measured the total plasma cholesterol concentrations in 25 primary *P knowlesi* infections in *M mulatta* and found very wide

He found the normal range of values in British soldiers and Indians lay between 121 and 192 mgm per cent plasma The observations on malaria cases were carried out in soldiers suffering from chronic infections When first examined these subjects had not had relapses or antimalarial treatment for some time Cholesterol estimations were carried out when the parasites appeared in the peripheral blood during the paroxysm in the immediate apyrexial period (12–18 hours after temperature had become normal) and after specific treatment (quinine and pamaquin) Estimations were made at all four times in 25 patients In all cases the cholesterol concentration was low (about 100 mgm per cent) immediately before the fever It was raised considerably during the fever (average about 210 mgm per cent) and fell to subnormal levels in the immediately post-pyrexial period becoming normal after treatment

In four benign tertian cases frequent observations were possible during the paroxysm In two of these the rise in cholesterol concentration occurred before the rigor commenced The maximum level was reached within three hours of the commencement of the paroxysm and the subsequent fall in each case preceded that of the temperature The author reports similar changes in two *P falciparum* infections

Total free and ester blood cholesterol concentrations were measured by Kopp and Solomon (1943) in the course of induced benign tertian malaria in nine neurosyphilitics The total cholesterol during the malaria fell considerably in all patients the mean being 126 mgm per cent compared to 219 mgm per cent before the onset (normal values according to these authors range from 140–230 mgm per cent) The free cholesterol also fell but there was an even more pronounced lowering of the cholesterol ester fraction The phospholipoids of the blood were also reduced Other signs of interference to liver function were also present in these cases

Ross (1932) estimated whole blood and plasma cholesterol concentrations in 10 cases of *P falciparum* malaria and found values ranging from 129–200 mgm per cent for the former and 102–160 for the latter According to Fairley s and Kehar s estimates for normal concentrations in whole blood Ross s results indicate no deviation from normal but according to the normal figures proposed by Kopp and Solomon for plasma cholesterol all except three showed some degree of *hypocholesterolaemia*

In 15 cases of blackwater fever Ross (1932) found the total whole blood cholesterol varied from 114–234 mgm per cent and the plasma cholesterol from 75–200 mgm per cent The lowest figures for each

were recorded in the same case. Fairley and Bromfield reviewed the findings of other workers and reported concentrations of 68–90 and 77–93 mgm per cent respectively in two fatal cases of blackwater fever. In three non-fatal cases the values ranged from 71–109 mgm per cent. Transfusion of blood made little difference to the cholesterol concentration. The average for 18 estimations in all five cases was 86.5 mgm per cent. There was thus a persistent hypocholesterolaemia.

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After termination of the malaria by quinine the cholesterol concentrations returned to normal or slightly higher values. During latent periods in the infection the cholesterol concentrations were just below normal, but prior to relapse they rose. During a relapse the cholesterol frequently fell lower than in the primary infection, and in long-standing cases the authors claimed that there was a state of persistent hypocholesterolaemia.

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Kehar (1937) measured the total plasma cholesterol concentrations in 25 primary *P. knowlesi* infections in *M. mulatta* and found very wide

deviations in individual animals although the mean value obtained was normal The average plasma cholesterol in a further 34 monkeys suffering from chronic *P knowlesi* *P inui* and *P cynomolgi* infections was 71 mgm per cent (Normal value 100 mgm per cent) Where changes in cholesterol had occurred quinine and plasmoquine therapy restored the normal levels in five to seven days More numerous estimations were made in 20 monkeys inoculated with *P knowlesi* The average cholesterol concentration before inoculation was 103 mgm per cent It remained the same during the incubation period and during overt infection averaged 99 mgm per cent The range of variation between the values recorded for different animals throughout the experiment was too great to allow any conclusions to be drawn from the results except that in animals which developed haemoglobinuria there was an abrupt appreciable rise in cholesterol concentration shortly before death Quantitative changes in cholesterol fractions were found in 25 blood samples examined The variations were a rise in the cholesterol esters and an almost proportionate fall in the free cholesterol The former observation is at variance with that of Kopp and Solomon in human malaria who noted a fall in both cholesterol fractions particularly in the esters

The phospholipoid content of the blood in human malaria has not received much attention from research workers but where estimations have been made the concentrations have been found to be low during an attack although Grieg *et al* reported normal values during the apyrexial interval Kopp and Solomon (1943) measured the total blood lipoid in nine neurosyphilitics during therapeutic benign tertian malaria and observed a decided fall in concentration Normal values were restored after termination of the malaria by quinine The lecithin content of the blood in blackwater fever was also found to be low in four cases out of six in which estimations were made by Whitmore and Roe (1929) during the attack and between 2 and 60 hours from the onset of haemoglobinuria

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fall during the patent disease period. In one animal which did not pass haemoglobin in the urine this decrease continued up to death in the other three which died in a haemoglobinuric state there was an abrupt rise to normal values before death. In two other monkeys which died without developing haemoglobinuria cholesterol estimations were made simultaneously with those of lecithin In both animals the concentration of cholesterol and lecithin fell steadily during the active disease period after a temporary fall followed by a sharp rise towards the end of the incubation period On the other hand in two other monkeys which did develop haemoglobinuria there was an initial fall in the concentrations of both substances during the overt infection followed by a sharp rise shortly before death at the time of the appearance of haemoglobinuria A similar rise in cholesterol concentration was as we have seen above recorded by Krishnan *et al* (1936) in some but not all haemoglobinuric cases of *P knowlesi* infections in *M mulatta*

It will be seen that the details of the findings of various workers differ considerably with regard to the concentrations of cholesterol obtaining in malaria but there is some agreement on several points In well-established malaria hypocholesterolaemia occurs in many cases but not in all. In primary infections results vary but both Crespin and Zaky and Kehar have noted hypercholesterolaemia during the paroxysm. The latter author found a pronounced increase in all his cases in the early stages of the paroxysm. When the total cholesterol is decreased the free fraction is reduced and according to Kopp and Solomon the ester fraction is also reduced Kehar on the other hand found the latter raised in monkey malaria.

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haemolysis or as a cure for prevailing haemolysis Success has been claimed by Ott (193-) but the administration of cholesterol has no effect on the incidence of haemoglobinuria in monkeys infected with *P knowlesi* (Kehar 1937)

INORGANIC CONSTITUENTS

Plasma potassium and sodium

Most of the potassium of the blood is held in the erythrocytes so that when these are destroyed rapidly for example in haemolytic anaemias excess potassium is liberated into the plasma A raised plasma concentration might therefore be expected in severe malaria when red cell destruction is pronounced for instance during the sporulation of the plasmodia This has been found by most observers to be the case but there is some doubt as to whether mere red cell destruction can account for the elevation of potassium concentration which occurs

Pinelli (19 9) measured serum potassium concentration in cases of *P vivax* and *P falciparum* malaria and recorded values ranging from -7 to 40 6 mgm per cent during the febrile period and a return to approximately normal figures during the apyrexial intervals (Normal concentration of plasma potassium is about 18 to -0 mgm per cent) In the same year Andriadse reported a very greatly increased concentration of plasma potassium during the paroxysms of *P vivax* *P falciparum* and mixed infections and a fall to below normal values in the apyrexial interval He observed rapid excretion of potassium after the peak concentrations had been reached

Velick and Scudder (1940) investigated the changes in plasma potassium concentration in *P cathemerium* infections in birds They found that in infected canaries with 4 to 14 per cent parasitaemia in which sporulation occurred mainly in the evenings the potassium concentrations ranged from -9 to 44 mgm per cent (-1 to 25 mgm per cent in uninfected birds) the higher values obtaining in the evenings Smaller changes were noted in chickens and ducks (infected with *P lophurae*) in the infections of neither of which was there any marked synchronicity These authors suggest that the source of the raised potassium in their birds may have been the erythrocytes since *P cathemerium* invaded almost exclusively immature red cells which contain more potassium than more mature cells (Henriques and Ørskov 1936)

Zwemer Sims and Coggeshall (1940) studied the plasma potassium concentrations in cases of induced *P vivax* malaria in paretics Concen-

Krishnan *et al* make the suggestion that the great variations in cholesterol concentrations seen in the early stages of malaria of monkeys may be due to failure of mobilization of cholesterol and possibly to inhibition of synthesis They suggest that cholesterol may be important for parasitic metabolism in some way What they mean is not very clear but low lecithin concentrations might well reflect incomplete synthesis Recent work has shown that in hepatectomized dogs after injection with radioactive phosphorus no radioactive phospholipoids appear in the plasma (Hevesy 1940) It would not be surprising therefore if in malaria which causes such severe disturbances of liver function synthesis was temporarily inhibited with a consequent reduction in plasma phospholipoid concentration

The liver damage in malaria is associated with intrahepatic anoxia and it is interesting to note that such anoxia gives rise to an increase in plasma lipoids Lipaemia has frequently been recorded in severe anaemic anoxia arising from haemorrhage in animals Starup (1934) claims that pronounced lipaemia can also be produced in rabbits by the administration of phenylhydrazine the destruction of corpuscles by intravenous injection of distilled water or the exposure of the animals to atmospheric oxygen lack No work on the effect of anoxia on blood lipoids has been done in the human subject and so far the animal experimentation has led to somewhat conflicting results It is not therefore possible to extend the argument to the human subject until further research has been carried out

Fairley and Bromfield have drawn attention to the possibility that there may be some common factor involved in the production of the hypocholesterolaemia seen in malaria and that in pernicious anaemia They point out that in the latter disease liver treatment restores the blood cholesterol concentrations to normal levels the return to normal keeping pace with the development of reticulocytosis In three of their cases Fairley and Bromfield observed a similar relation between the restoration of cholesterol concentration and an increase in reticulocytes during treatment

The relation between the concentration of cholesterol and the appearance of haemoglobinuria has not been ascertained Fairley and Bromfield point out in this connection that information concerning the concentration at the time of severe lysis is required before the problem can be solved and no knowledge of this is available It is known that cholesterol in excess will inhibit some forms of haemolysis (Ponder 1944) and with this in mind some workers have tried the clinical effects of the administration of cholesterol as a preventive of

-- 6 mgm in one monkey -8 9 and -- 0 in the other during the afternoon and morning respectively) Similar results were obtained in a third animal in which the blood was taken on the day before the appearance of parasites The concentrations were raised in both morning and evening plasma samples in a fourth infected monkey More definite peak concentrations were found in two infected animals which were bled at more frequent intervals In one of these (bled at half hourly intervals) a value of 40 mgm per cent was reached the maximum concentration corresponding to the peak period of sporulation The rise in potassium concentration at this period was very rapid and there was a subsequent rapid fall As the disease progressed the potassium concentration of the whole blood began to fall the decrease being roughly proportional to the loss of red cells

Velick and Scudder as a result of their experiments in bird malaria suggested that the chief source of the excess potassium in the plasma was probably the erythrocytes haemolysed during the disease particularly as the parasitized cells were mostly immature and therefore high in potassium content To support their view they pointed out that similar increases in potassium concentrations have been reported in severe haemolytic anaemias Moreover Colale (1930) found that if rabbit red cells were lysed *in vivo* an increase in plasma potassium developed roughly in proportion to the red cells destroyed Elevations of plasma potassium have also been observed in experimental *Trypanosoma equiperdum* infections shortly before death at a time when there is considerable destruction of erythrocytes (Zwemer and Culbertson 1939)

Zwemer Sims and Coggeshall however made an estimate of the total quantity of potassium available in the cells lysed in cases of malaria and found that the amount available was insufficient to account for the concentration in the plasma especially during the early stages of the paroxysm They therefore came to the conclusion that some of the excess potassium must come from tissue cells and suggested that such release of intracellular potassium might originate from either the toxic influence of the excess of that ion or from generalized cellular damage caused by high fever

There is nevertheless some relation between the destruction of the parasitized cells and the prevailing potassium level since Zwemer *et al* and Flosi found that the peak plasma concentration coincided with the beginning of the paroxysm in man and with the period of maximal sporulation in monkeys It has therefore been suggested that some reaction of an anaphylactoid nature may develop during this

trations were measured immediately after inoculation with trophozoites during the incubation period and after the onset of symptoms Once the malaria was established the plasma potassium concentration was found to rise to an average highest value of 35 2 mgm per cent The peak concentration occurred usually at about the time of the beginning of the rigor and often preceded the rise in temperature As the temperature rose the potassium concentration fell steadily reaching a fairly constant level before the end of the paroxysm During the clinical activity of the malaria the concentration nearly always exceeded the normal values for the individual patient It was occasionally normal during the afebrile period There were no corresponding changes in potassium concentrations in control cases not treated with malaria

Flosi (1944) during an investigation of the suprarenal functions in human malaria measured the plasma potassium and sodium concentrations in 12 cases of *P vivax* *P falciparum* and mixed infections In most of these patients he found raised potassium and lowered sodium concentrations during the attack and immediately after quinine therapy In two cases of *P vivax* malaria the concentrations of these ions were measured before during and after a paroxysm His results in these cases were substantially the same as those of Zwemer *et al* In the first of these patients nearly two days before the rigor the plasma potassium and sodium concentrations were 19 and 324 mgm per cent respectively On the day before the rigor when schizonts were appearing the concentrations were 18 5 mgm and 325 mgm per cent During the rigor when the schizonts were rupturing the concentrations were 24 mgm and 293 mgm After the fever fell the values were 18 5 mgm and 320 mgm respectively Quinine therapy was commenced and the following day the concentrations of potassium and sodium were about the same as before the rigor In the second case very similar figures were obtained In both the rise in potassium preceded that of the temperature For example in the second case the potassium level had fallen from a peak value of 27 0 mgm to 24 0 mgm per cent before the maximal temperature was reached

Zwemer Sims and Coggeshall also examined the plasma potassium concentrations in monkeys (*M mulatta*) infected with *P knowlesi* No marked periodicity was observed in these infections but when the specimens of blood were taken morning and afternoon it was found that in two infected animals the afternoon specimen contained considerably more potassium than the morning sample (28 6 mgm and

22 6 mgm in one monkey 28 9 and 2 0 in the other during the afternoon and morning respectively) Similar results were obtained in a third animal in which the blood was taken on the day before the appearance of parasites The concentrations were raised in both morning and evening plasma samples in a fourth infected monkey More definite peak concentrations were found in two infected animals which were bled at more frequent intervals In one of these (bled at half-hourly intervals) a value of 40 mgm per cent was reached the maximum concentration corresponding to the peak period of sporulation The rise in potassium concentration at this period was very rapid and there was a subsequent rapid fall As the disease progressed the potassium concentration of the whole blood began to fall the decrease being roughly proportional to the loss of red cells

Velick and Scudder as a result of their experiments in bird malaria suggested that the chief source of the excess potassium in the plasma was probably the erythrocytes haemolysed during the disease particularly as the parasitized cells were mostly immature and therefore high in potassium content To support their view they pointed out that similar increases in potassium concentrations have been reported in severe haemolytic anaemias Moreover Colale (1930) found that if rabbit red cells were lysed *in vivo* an increase in plasma potassium developed roughly in proportion to the red cells destroyed Elevations of plasma potassium have also been observed in experimental *Trypanosoma equiperdum* infections shortly before death at a time when there is considerable destruction of erythrocytes (Zwemer and Culbertson 1939)

Zwemer Sims and Coggeshall however made an estimate of the total quantity of potassium available in the cells lysed in cases of malaria and found that the amount available was insufficient to account for the concentration in the plasma especially during the early stages of the paroxysm They therefore came to the conclusion that some of the excess potassium must come from tissue cells and suggested that such release of intracellular potassium might originate from either the toxic influence of the excess of that ion or from generalized cellular damage caused by high fever

There is nevertheless some relation between the destruction of the parasitized cells and the prevailing potassium level since Zwemer *et al* and Flosi found that the peak plasma concentration coincided with the beginning of the paroxysm in man and with the period of maximal sporulation in monkeys It has therefore been suggested that some reaction of an anaphylactoid nature may develop during this

phase of the plasmodial cycle resulting from the sensitization of the body in some way to the discharge of the final products of schizogony into the general circulation An increase of plasma potassium concentration has been observed in anaphylactic shock in dogs and rabbits (Schuttenhelm Erhardt and Warnat 1928) and it is now well established that plasma potassium is also elevated in other forms of shock including surgical secondary shock sometimes to double or more the normal values High levels of potassium however also occur in certain related phenomena which may conceivably play an important part in the general picture of malaria Thus elevation of plasma potassium associated with a sharp fall in sodium concentration and a reduction in total plasma alkali results from adrenal cortical insufficiency (or adrenalectomy) and factors which disturb the blood electrolyte balance may produce corresponding changes in adrenal cortical function A similar rise in plasma potassium also occurs in certain forms of anoxia such as asphyxia and severe anaemia The high potassium concentrations developing after asphyxia and after experimental haemorrhage are connected in some way with the adrenal gland and the liver since the potassium rise in the former can be inhibited by adrenalectomy and the rise in both cases can be prevented by hepatectomy (Mullin Dennis and Calvin 1938 Houssay Marenzi and Gerschman 1936 1937 Fenn 1940) There is still a division of opinion on this point but most workers are agreed that the initial increase in potassium (and the corresponding rise in blood sugar) follows stimulation of the adrenal medulla to produce adrenalin which in turn mobilizes the potassium from the liver cells and possibly from the heart and skeletal muscles It appears therefore that the anoxic processes associated with malaria such as the reduction of numbers of red cells and changes in blood circulation through the organs (see Chapters on liver kidney etc) are probably concerned to some extent in the production of the high plasma potassium concentrations met in the disease It is interesting to record that anoxia may itself cause active damage to the adrenals and so aggravate the process It has been shown for instance that anoxic anoxia deriving from deficiency of atmospheric oxygen may give rise first to hypertrophy and later to degenerative changes in the adrenals especially the cortex (Armstrong and Heim 1938 Emerson and van Liere 1938) Clinical and pathological evidence of changes in the adrenals will be discussed later There is much in the clinical picture of malaria that may be referable to adrenal insufficiency and the coexistence of such clinical evidence with raised plasma potassium levels indicates that the latter may owe their origin in part to adrenal dys-

function There is evidence also that raised potassium concentrations may react adversely on the adrenals so that a vicious circle is sometimes set up

High plasma potassium concentrations are in themselves extremely toxic and may be fatal they are difficult to maintain experimentally The values obtaining in malaria and shock are however much below the lethal level and are unlikely in themselves to cause death (Manery and Solandt 1941) It is not now considered that such concentrations are of primary or major importance in the development of shock states

Most observers have noted very little change in the calcium content of blood in malaria and blackwater fever (Schittenhelm *et al* 1928 Andriadse 1929 Fairley and Bromfield 1934 Whitmore and Roe 1929 Wats and Das Gupta 1934) It follows therefore that the changes in potassium concentration will bring about rapid fluctuations in the potassium/calcium balance Several authors have suggested that restoration of this balance to normal might minimize the anaphylactic state and Schittenhelm *et al* proposed the administration of calcium with this object in view Zwemer *et al* and Flosi have clearly shown the relation of the peak potassium values to the beginning of the paroxysm and consider that this association of high potassium and rigor may explain the effect of calcium chloride in terminating the chill period (Beeson and Hoagland 1940)

Chlorides

The chloride content of the blood has not been closely observed in malaria although several workers have recorded measurements during the course of blackwater fever In both conditions it is apparently often lowered Miyahara (1936) investigated the plasma and whole blood chloride content in over 100 cases of malaria (presumably benign tertian) and observed a reduction in the former during the febrile period of the disease There was actually a small rise in erythrocyte chloride concentration and the author suggested that this might be due to some change in permeability of the erythrocyte cell membrane possibly associated with acidosis Lahille (1915) examined the serum chlorides (expressed as sodium chloride) in two cases of blackwater fever In a fatal case dying in anuria there was a very great reduction on the day before death and a steady fall in concentration prior to that In the second and non-fatal case the concentration remained within normal limits Wakeman (1929) found the serum chlorides much reduced in a non fatal anuric case especially over the period of the anuria and the beginnings of renal recovery The same

author with Morrell (1929) reported normal chloride concentrations Ross (1932) found the chloride content of the blood in his cases of blackwater fever varied considerably it was greatly diminished in some cases Fairley and Bromfield (1934) in a fatal case dying from renal acidosis reported somewhat low plasma chlorides (528 mgm per cent)

The significance of the low chloride content in relation to the renal function will be discussed elsewhere

Other ions

No gross deviations from normal in the content of inorganic phosphate have been reported in malaria (Wats and Da Gupta 1934 Ross 1932) In blackwater fever however it is frequently found elevated particularly in those cases which present clinical features suggesting acidosis Fairley and Bromfield (1934) for instance found a concentration as great as 10 7 mgm per cent in a fatal case with raised blood urea low blood chlorides and a very low plasma carbon dioxide combining power These authors also observed raised plasma inorganic phosphate in *P knowlesi* infections of *M mulatta* Ross (1932) found raised inorganic phosphate in only two severe cases of blackwater fever Phosphatase has been measured only in relation to liver function tests and in these as far as can be ascertained only in chronic relapsing *P vivax* cases in which it is apparently unchanged (Lippencott *et al* 1945) Other inorganic constituents of blood with the exception of those mentioned below in reference to the physicochemical properties of blood in malaria do not seem to have received any attention

ALKALI RESERVE

Many workers have attempted to demonstrate a reduction in alkali reserve in malaria and blackwater fever Sinton and Baily (1924) observed the amount of sodium bicarbonate administered orally which was necessary to make the reaction of the urine alkaline in normal Indians and patients with *P vivax* and *P falciparum* infections They found that whereas on an average normal subjects passed alkaline urine after only 6 5 gm of sodium bicarbonate had been administered patients suffering from benign tertian malaria needed 13 9 gm and those suffering from malignant tertian 14 5 gm of bicarbonate to produce the same effect They suggested that these results indicated a diminution of alkali reserve and that there was possibly therefore some acidosis present in the malarial cases The degree of reduction in alkali

reserve was greater in the more severe malignant tertian infections Hinrichs (1926) found a reduction in alkali reserve in malaria but was unable to confirm Sinton's observation that a relation existed between the degree of acidosis and the severity of the case Blood alkali was also lowered in fever from other causes and Hinrichs put forward the view that the raised temperature of malaria might be responsible for the reduction in alkali reserve This has not been confirmed by Bischoff Long and Hill (1931) who showed that in artificially induced hyperthermia there was a tendency not to acidosis but to alkalosis presumably resulting from hyperventilation frequently associated with the passage of an acid urine and sometimes with an increase in blood pH High alkali reserves have in fact been recorded by Sirca (1929) in chronic malaria associated with an enlarged spleen in children In acute cases in children this author observed a reduction in alkali reserve particularly during the apyrexial period

There are many theoretical objections to the method used by Sinton and Baily to determine alkali reserve Bicarbonate tolerance tests of this kind are of use only when the kidney is functioning normally and any disturbance of renal function may interfere with the bicarbonate excretion and upset the results of the test by indicating a lower reserve than in fact obtains This applies particularly in malaria More direct methods are required for determination of the alkali reserve in disease and where these have been applied the results indicate that in uncomplicated malaria there is little change in the bicarbonate content of the blood Ross (193-) found the plasma carbon dioxide in acute and convalescent malignant tertian malaria lay within normal limits Fairley and Bromfield (1933) reported similar results in seven cases of benign tertian (average carbon dioxide 60 7 vols per cent) and 16 cases of malignant tertian malaria (average carbon dioxide 60 6 vols per cent) In two cases of induced benign tertian malaria these authors found no change in plasma carbon dioxide but in a third case which was suffering from intercurrent renal disease the value fell frequently below 50 vols per cent and on one occasion to 38 vols per cent They consider their results indicate that there is no evidence of significant reduction of alkali reserve in uncomplicated malaria

More conflicting results have been obtained in blackwater fever in which under certain conditions the alkali reserve may be considerably reduced Wakeman (1929) and Wakeman and Morrell (1929) recorded no change in plasma carbon dioxide in one non fatal anuric case and in a case in which the estimates were made during

haemoglobinuria Wakeman *et al* reviewing these cases state that alkalosis followed bicarbonate administration and consequently there can have been no fall in alkali reserve but possibly a rise resulting from excessive vomiting Fairley and Bromfield quote Whitmore (1928) who recorded values of under 50 vols per cent in two cases one of which showed no evidence of nitrogen retention Whitmore and Roe (1929) report a plasma carbon dioxide concentration of 35 vols per cent in a third case on the second day of the disease and values of 49 and 48 5 vols per cent in two others These authors consider that only the first of these cases exhibited a significant reduction of plasma alkali reserve Ross (1932) examined the plasma bicarbonate concentration in four active and three convalescent cases of blackwater fever and found no evidence of reduction He considered therefore that there was no acidosis in his cases but he did not deny that such a state could exist in some patients as ketonuria was sometimes recorded in malaria

Fairley and Bromfield (1934) investigated seven cases of blackwater fever In three fatal cases all exhibiting urea nitrogen retention plasma carbon dioxide concentrations of under 50 vols per cent were observed In one case four hours before death the concentration was only 21 8 vols per cent and was associated with the very high inorganic phosphorus level of 10 7 mgm per cent The latter patient died according to the authors in a state of uncompensated acidosis possibly renal in origin in view of the passage of very acid urine containing no ketone bodies This patient was given bicarbonate orally but absorption was largely prevented by vomiting The other two fatal cases were given alkali orally and per rectum and by intravenous injection respectively In two non-fatal cases in which there was no concomitant increase in blood urea nitrogen the plasma carbon dioxide concentration was found normal In a third case the blood urea nitrogen was moderately raised and the plasma carbon dioxide was 48 0 vols per cent on the third day In the fourth non-fatal case the plasma carbon dioxide fell from 52 4 vols per cent to 33 1 vols per cent in the course of a few hours on the fourth day of the illness with an accompanying increase in inorganic phosphorus and the passage of acid urine Administration of bicarbonate restored the plasma carbon dioxide to 60 3 vols per cent The authors consider that renal acidosis was developing in this patient and was relieved by the administration of

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there is a concomitant rise in blood urea nitrogen Fairley and Bromfield (1934) consider that acidosis of renal origin may arise in some of these cases They very rightly point out however that although reduction of alkali reserve may usually be taken to represent the appearance of acidosis this is not always the case since alteration of the proportion of free carbon dioxide to combined carbon dioxide will affect the buffering and ultimately the reaction of the blood Thus Muntwyler Limbach Bill and Myers (1931) found a lowered alkali reserve in cases of toxaemia of pregnancy associated with an *elevated* blood pH and a reduction in total base They considered that the reduced plasma bicarbonate was the result of hyperventilation and the fall in total base therefore compensatory

Actual pH changes in malaria do not seem to have been recorded except in *P knowlesi* infections in monkeys (*M mulatta*) In three infected monkeys all of which died in a haemoglobinuric state Kehar (1936) observed some fall in pH following the appearance of parasites in the peripheral blood and a very appreciable fall in the terminal stages of the disease Kehar states in the summary of his paper that the pH values were slightly affected when the parasitic infection is very heavy In view of a fall in pH in one animal from the normal (7 328–7 389) to 7 055 on the day before death, this must be regarded as an understatement Confirmation of these changes is needed before they can be accepted since large deviations of pH indicate most severe derangement of acid-base balance of the blood (Harkins and Hastings 1931)

OTHER CHANGES IN THE BLOOD

Sinton Orr and Ahmed (1928) reported a rise in refractive index of the serum in human malaria during the rigor and a fall to normal values after the paroxysm and during the apyrexial period A rise in this index has been recorded in anaphylactic shock (Zunz and La Barre 1922) Kehar (1936) found a slight increase during the active disease in *P knowlesi* infection in three monkeys (*M mulatta*)

Changes in serum surface tension have also been reported in human malaria during the paroxysm and in *P knowlesi* infections of monkeys No variations in the specific gravity of the blood have been recorded (Kehar 1936)

The significance of the above findings is obscure but they are probably related to the changes which occur in the plasma proteins during the infections (see section of blood protein)

The changes in the cellular constituents of the blood are dealt with elsewhere (Chapter III)

The variations of urea nitrogen in the blood in malaria and blackwater fever are discussed in Chapters VII and VIII

BLOOD PIGMENTS

Barratt and Yorke (1909) made the first quantitative measurements of the haemoglobin concentration in the plasma of malaria patients They found no significant rise in 14 cases Fairley and Bromfield (1933) examined the plasma of 32 cases (21 *P falciparum* 10 *P vivax* and one *P ovale* infections) and found traces of haemoglobin in 14 ranging from 0 03 to 0 12 per cent (estimated as percentage of normal blood) Methaemoglobin was never present The plasma of one of two patients infected artificially with *P vivax* showed traces of haemoglobin during fever Traces of haemoglobin were however noted in seven of 53 normal controls (ranging from 0 07 to 0 12 per cent) The authors suggest that in malaria the haemoglobin of the parasitized red cells may be destroyed or metabolized into malarial pigment before the destruction of the cell (Christophers and Fulton have recently (1938) demonstrated the disappearance of active haemoglobin from invaded cells in *P knowlesi* infections of *M mulatta*) The absence of significant amounts of haemoglobin in the plasma may also partly depend on the phagocytosis of whole parasitized erythrocytes by the cells of the reticulo-endothelium

Many investigators have recorded the presence of haemoglobin in the serum or plasma of cases of blackwater fever Relatively few however have made quantitative estimations (Christophers and Bentley 1908 Barratt and Yorke 1909 Thomson 1924 Ross 1927) Owen and Murgatroyd (1928) summarized the literature to that date and Yorke Murgatroyd and Owen (1930) reported the results of quantitative measurements of haemoglobinaemia in five cases of blackwater fever (including two cases previously reported by Owen and Murgatroyd) *in all of which haemoglobin was found in the plasma* during and for a variable time subsequent to the passage of haemoglobin in the urine These authors noted in one case that there was a close relation between the amount of haemoglobin in the plasma (estimated as percentage of normal blood) and the prevailing haemolysis (measured in terms of the fall of erythrocyte count and the intensity of the haemoglobinuria) Thus on the third day after the red cell count had fallen from 2 8 to 1 6 million cells per cu mm in 24 hours

the total haemoglobinaemia was 1 1 per cent The value for the previous day during which the red cell count fell from 3 4 to 2 8 million cells per cu mm was 0 44 per cent and for the succeeding day during which the red cell count remained unaltered 0 25 per cent On the fifth day the first day free from haemoglobinuria the haemoglobinaemia was 0 13 per cent and on the seventh day had disappeared In another case the highest values were recorded during the haemoglobinuric phase being 2 1 per cent and 1 25 per cent on days one and two respectively haemoglobin was still present in the plasma 10 days after it had disappeared from the urine The authors considered that this may have been associated with the temporary renal failure which developed

Yorke Murgatroyd and Owen recorded the presence of methaemoglobin in two of their cases and noted that this pigment was present in the plasma but not in the corpuscles themselves Ross (1932) later confirmed the presence of this pigment mixed with haemoglobin in 12 of 18 cases examined

Yorke and his colleagues considered that in view of the degree of haemolysis which took place so rapidly in their cases the concentration of haemoglobin observed was much smaller than might be expected They therefore suggested that the haemoglobin from the lysed cells entered the circulation more slowly than in other haemolytic states e g *Babesia* infections in dogs and concluded that haemolysis probably occurred mainly in the organs such as the spleen and liver and not in the peripheral blood

Fairley and Bromfield (1933) studied the problem in nine cases of blackwater fever in five of which serial estimations were made They measured the true plasma concentrations of oxyhaemoglobin bilirubin and methaemoglobin In those cases in which serial observations were made these workers found that haemoglobinaemia was present in all in two the values were distinctly higher than those recorded by previous workers e g a haemoglobinaemia of 5 to 14 per cent was recorded in a fatal case They considered that the amount of pigment present was enough to explain the phenomena of blackwater fever in terms of an intravascular haemolysis and that there was thus no need to postulate haemolysis in the spleen etc as suggested by Yorke *et al* Methaemoglobin was present in all cases except one in which a related but entirely new pigment was demonstrated (methaemalbumin) Methaemoglobin was found to be the predominant pigment in the plasma it was not found in the corpuscles confirming the findings of Yorke *et al* (1930)

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stored in the phagocytic cells of the reticuloendothelium and only very slowly excreted The method of excretion is not known It does not appear to be an intermediate product in the synthesis of bile pigments (Duesberg 1934) Rigdon (1945) has suggested that in duck malaria there may be some slow oxidation of the haematin into haemosiderin which could be used by the host

The haemoglobin liberated into the plasma by lysis of both parasitized and unparasitized cells is removed from the circulation rapidly In severe haemolytic states a small proportion may pass through the kidneys and appear in the urine but the bulk of the pigment is either absorbed into the cells of the reticuloendothelial system or split and synthesized into the new pigment methaemalbumin The haemoglobin taken up by the reticuloendothelial cells is converted into bilirubin via the production of biliverdin formed by the opening of the protoporphyrin ring by partial oxidation and subsequent loss of both iron and protein The bilirubin is excreted through the liver and appears in the plasma when there is excess production or some interference with its excretion Some of the haemoglobin however is converted into methaemalbumin whenever there is excess for a sufficient length of time to permit the synthesis The oxyhaemoglobin is broken down to globin and haematin the latter uniting with crystalbumin to form methaemalbumin the molecule of which is too large to pass through the kidneys and escape into the urine The subsequent fate of the methaemalbumin is not certain but Rimington (1939) has shown that an increased faecal excretion of porphyrin follows intravenous injection of haematin in man monkeys and rabbits This increased excretion persists for longer in man and monkeys than in the rabbit which does not form methaemalbumin He suggests that the methaemalbumin is removed via the liver after a process in which the pigment is converted not into bile pigments but into porphyrin Fairley (1939) points out that if this were so methaemalbumin should be persistent in cases in which there was derangement of liver function He states that he has confirmed this clinically in cases of hepatic cirrhosis associated with haemolytic anaemia and haemoglobinuria

Rimington's observation that intravenous injection of haematin leads to increased faecal excretion of porphyrin indicates the probable route of the break-down of malarial pigment Fairley (1938) has shown that methaemalbumin is synthesized *in vivo* from injected haematin or large quantities of haemoglobin so that it is possible that the malarial pigment itself haematin may be finally converted into methaemalbumin and broken down and excreted as porphyrin through the liver

Fairley and his co-workers found that although methaemoglobinuria is common in blackwater fever true methaemoglobinaemia does not occur The circulating pigment described in earlier work as methaemoglobin was in fact, the pigment first identified by Fairley and Bromfield (1934) and subsequently named by the former methaemalbumin

This pigment was related to methaemoglobin but differed from it in certain important chemical properties e g its protein fraction was albumin and not globin It never appeared in the urine and was not found in the red cells *It was first called pseudomethaemoglobin* but the name was changed later (Fairley 1938) to methaemalbumin since it was found that it could be synthesized from haematin and crystalbumin (the latter from human or monkey serum but not from rabbit serum) Keilin (1944) has recently suggested that it should be called haematin-albumin when the iron is in the trivalent state She has also produced evidence suggesting *that the linkage of the albumin to the haematin is not to the iron* but to the porphyrin Methaemalbumin gives a positive reaction with Schumm s reagent which enables it to be distinguished from haematin and oxyhaemoglobin Its molecule is too big to pass through the kidneys into the urine It is unable to function as a respiratory pigment (Fairley 1938 1939)

Methaemalbumin can be synthesized *in vivo* by injection into man *or monkeys of alkaline haematin Fairley (1939) has also found* it present 6 to 12 hours after heavy doses of haemoglobin intravenously It has been identified constantly in severe cases of blackwater fever and in some other conditions including incompatible blood transfusion nocturnal haemoglobinuria and certain anaemias not associated with haemoglobinuria it is not present in myohaemoglobinuria

The fate of the haemoglobin of the parasitized cell in malaria has been determined to some extent by the recent work of Devine and Fulton (1941) Christophers and Fulton Morrison and Anderson (1942) and others There is little doubt that a large proportion of the pigment is converted into haemozoin before the rupture of the cell and the completion of schizogony This process apparently requires the splitting of the globin from the molecule and the retention of the *iron-haematoporphyrin* nucleus (haematin) which according to Morrison and Anderson (1942) may be present either as free haematin or preformed ferrihaemic acid In this state the pigment remains in the plasmodia until these are destroyed and phagocytosed It is then

stored in the phagocytic cells of the reticuloendothelium and only very slowly excreted The method of excretion is not known It does not appear to be an intermediate product in the synthesis of bile pigments (Duesberg 1934) Rigdon (1945) has suggested that in duck malaria there may be some slow oxidation of the haematin into haemosiderin which could be used by the host

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CHAPTER V

THE LIVER IN MALARIA AND BLACK-WATER FEVER

(i) *Clinical evidence of hepatic dysfunction*

JAUNDICE BILIRUBINAEMIA THE INTERPRETATION OF THE INDIRECT VAN DEN BERGH REACTION BILE PIGMENTS IN THE URINE LIVER FUNCTION TESTS

SIGNS and symptoms of disturbance of liver function may appear during the course of all forms of human malaria both naturally acquired and artificially induced The degree of liver damage varies considerably from case to case and is dependent to some extent on the species of invading plasmodium and the severity of the infection

The most serious disturbances have been reported in *P falciparum* infections in which the development and progress of hepatic failure sometimes associated with evidence of polycholia may completely dominate the clinical picture Extreme hepatic failure is seen in the syndrome usually called bilious remittent fever a form of pernicious malaria which is now uncommon but which was once greatly dreaded especially on the West Coast of Africa In the classical syndrome as described in text-books (Manson-Bahr) signs of liver failure appear early Severe gastro-intestinal disturbances develop rapidly including diarrhoea which is sometimes bilious and haemorrhagic and vomiting frequently bile stained and often intractable Jaundice develops during the first or second day and deepens rapidly bile pigments appear in the urine The liver enlarges and becomes extremely tender The condition is progressive and fatal In this syndrome the malarial attack appears to concentrate mainly on the liver but Fairley has pointed out (1932) that there is another type of bilious remittent fever in which the liver damage is not so obvious and which is characterized chiefly by haemolytic jaundice and a biphasic van den Bergh reaction with urobilinogen but not bilirubin in the urine

In the above conditions there is usually a high degree of parasitaemia Evidence of liver damage however may appear when the degree of invasion of the red cells is slight or when parasites are undetectable in the peripheral blood Deepening jaundice associated with bilirubinuria enlargement and tenderness of the liver and other signs of

liver involvement are not uncommon in blackwater fever in which a clinical picture very similar to that of classical bilious remittent fever may develop As in malaria, the jaundice of blackwater fever may be primarily haemolytic in origin but as we shall see later liver damage of the severest type may supervene in the disease (Ross, 193– Stephens 1937)

Signs of liver damage are also observed in uncomplicated malaria. Enlargement and tenderness of the liver sometimes associated with jaundice and bilirubinuria have for instance been frequently reported. Henry (193–) takes the extreme view that the liver is always affected in malaria, but this contention is not widely supported. Nevertheless according to Rudolf (19––) and Kern and Norris (1944) some signs of liver damage are common in all forms of human malaria, and at all stages of the disease whether in the primary attack or in subsequent relapses. Orlina also reported signs of liver dysfunction, especially in benign tertian infections, in about 50 per cent of her cases and states that enlargement of the liver is as frequent in malignant tertian and benign tertian malaria as is enlargement of the spleen. Many other observers have noted frequent evidence of liver involvement in malaria, but some e g Hughes and Bomford (1944) have found such signs uncommon, even in large series of cases.

Jaundice in malaria and blackwater fever

The jaundice observed in naturally acquired malaria is usually mild and is seen mainly as slight icterus of the conjunctiva and less commonly of the skin. Occasionally however it may be severe although the clinical picture is not so definite as that of bilious remittent fever Hughes and Bomford (1944) for instance reported, among over 800 cases of malignant tertian malaria six patients with jaundice and clay-coloured stools and bilirubinuria, and described the picture as indistinguishable from infective hepatitis Klotz reported jaundice in 10 of 1– fatal cases of malignant tertian malaria in Nigeria, eight with well marked jaundice and three with definite necrosis of the liver Ten out of 71 fatal cases of malignant tertian malaria dying between 19–5–4– in the Canal Zone had jaundice as a presenting sign (Kean and Smith, 1944)

Jaundice has frequently been observed during the course of malaria induced for therapeutic purposes James Nicol and Shute (193–) found it a common feature of therapeutic malignant tertian malaria, and Bunker and Kirby noted a mild degree of jaundice appearing usually between the fifth and eighth paroxysms in about a third of

their 53 cases of induced benign tertian malaria Many other authors have recorded mild jaundice in both induced *P falciparum* and *P vivax* infections sometimes in as many as half the cases and sometimes infrequently (Kopp and Solomon 1943 Fredricks and Hoffbauer 1945 O Leary Greene and Rowntree 1929 Wile and Sams 1934 Mirsky von Brecht and Williams 1944 McCorkle 1944)

Severe and increasing jaundice associated with enlargement and tenderness of the liver has also been reported in induced malaria Moore (1941) described several cases of what he called toxic hepatitis characterized by increasing jaundice and enlargement of the liver occurring in syphilitics during the course of therapeutically induced malaria Similar cases have been reported from time to time by other authors ever since malaria therapy was introduced (O Leary *et al* 1929 Fredricks and Hoffbauer 1945) The consistently short period between inoculation of the malaria and the appearance of jaundice excludes the possibility of syringe jaundice in these cases

Clinical signs of liver dysfunction also frequently occur in blackwater fever as already pointed out For instance in 46 fatal cases of blackwater fever seen in the British West African Colonies during 1941–43 jaundice was observed in 33 and of these the liver was enlarged in 12 and bile pigments were found in the urine in seven The significance of such findings and similar observations of other authors will be discussed later (Ross 1927 1932 Yorke Murgatroyd and Owen 1930 Owen and Murgatroyd 1928 Fairley and Bromfield 1934)

Jaundice appearing in malaria unaccompanied by bilirubinuria is according to many authorities nearly always haemolytic in type (McNee 1923) It is the result of the overproduction of bilirubin due to the excessive rapid haemolysis initiated by the infection to a point beyond the excretory capacity of the liver Retention of bilirubin in such cases may not therefore necessarily indicate any hepatic dysfunction in fact most authors appear to take it for granted that the excretory powers of the organ remain undisturbed Rich (1930) does not however agree with this and believes that there is an hepatic element in all forms of jaundice whether accompanied or not by bilirubinuria He points out that the normal excretory powers of the liver with regard to bilirubin have an enormous reserve (McMaster and Rous 19_1) and that conditions such as haemolysis which give rise to excessive production of bilirubin are practically always associated with some impairment of hepatic excretory function (This contention is borne out to some extent by the recent work on hepatic

function tests in malaria which is discussed below) In his view the appearance of jaundice in any given case depends on the balance existing between the bilirubin being produced and presented to the liver for excretion and the capacity of that organ to excrete it The latter is normally so great that retention of bilirubin occurs only when there is some reduction of the hepatic excretion function

Jaundice in malaria accompanied by bilirubinuria is usually associated with clinical signs of hepatic involvement such as enlargement and tenderness of the liver Here it is partly hepatogenous and reflects some degree of interference with biliary excretion by the liver and possibly damage to the polygonal cells

Bilirubinaemia

The nature of the jaundice appearing in malaria and blackwater fever has been investigated by many workers by estimating the serum bilirubin concentrations by the van den Bergh technique

Most authors agree that in uncomplicated naturally acquired malaria the direct van den Bergh reaction is only very rarely positive whereas the indirect reaction is commonly positive (indicating an elevation of circulating serum bilirubin) during the active stage of the disease returning to normal rapidly upon the subsidence of the infection or after treatment The serum bilirubin is not raised in latent malaria or mild attacks in which there is little fever and there are very few parasites in the peripheral blood (Kingsbury 1926 Schachsuwarly 1927 etc)

The indirect van den Bergh reaction has also been found positive in induced malaria Some authors have also reported positive direct reactions O Leary Greene and Rowntree for instance report a positive direct reaction in six syphilitics given malaria therapy associated with strongly positive indirect reactions of from 3 0 to 23 4 units bilirubin These reactions developed in the individual cases within 10 to 15 days of the onset of clinical symptoms of fever Fredricks and Hoffbauer (1945) have recently found both types of van den Bergh reactions present in therapeutic malaria They carried out quantitative bilirubin determinations in 31 syphilitics receiving malaria treatment and found significantly raised serum bilirubin (total bilirubin measured by the method of Malloy and Evelyn 1937) in nine in eight of which the direct reaction was positive Such findings differ considerably from those in natural uncomplicated malaria in which the direct reaction is seldom positive It is possible that syphilitic changes in the liver may sometimes explain the reactions observed in induced malaria

In blackwater fever most observers have recorded an increase in the indirect van den Bergh reaction The findings in regard to the direct reaction vary considerably Ross (1927) examined a series of 21 cases and found quantitative values for the indirect reaction ranging from 5 2 to 59 units In three fatal cases all of which exhibited suppression of urine the direct reaction was positive in all the other cases it was negative Owen and Murgatroyd (1928) reported two cases in which the indirect reaction was increased and the direct reaction positive on the eighth ninth and fifteenth day in one case and on the third and seventh day of the disease in the other Yorke Murgatroyd and Owen (1930) reported a further case in which the direct reaction as well as the indirect was positive between the first and twelfth days of the disease All these cases were jaundiced but in none of them was bile detected in the urine Owen and Murgatroyd pointed out that the three cases in Ross s series giving positive direct reactions were also the only ones examined after the first day (so far as can be judged from his tables) and that it is possible that some of the other cases might have developed similar reactions had they been further examined This is not supported by the evidence brought forward by Fairley and Bromfield (1934) who investigated seven cases of blackwater fever at intervals over periods ranging from 2 to 20 days and reported that none of these cases showed a direct positive reaction at any time although one showed a biphasic reaction On the other hand during the active stages of the disease they all had raised serum bilirubin indicated by the indirect van den Bergh reaction

The interpretation of the indirect van den Bergh reaction

Other things being equal the degree of bilirubinaemia in malaria is not apparently closely dependent on the species of invading plasmodium Kingsbury (1926) found that the average concentration of serum bilirubin was least in quartan (0 75 units) and greatest in malignant tertian (2 0 units) with benign tertian malaria in between (1 21 units) Ross (1927) obtained higher figures for malignant tertian but Wolski and Schewelewa (1929) found little difference between the average concentrations reached in the three infections (1 34 for quartan 1 30 for malignant tertian 1 2 for benign tertian) and Fairley and Bromfield (1934) reported that in their series of 30 malignant tertian and 20 benign tertian cases the average values reached were 1 45 and 1 75 units respectively

According to Wolski and Schewelewa (1929) the hyperbilirubinaemia of malaria is not always related to the degree of parasitaemia

Thus in one case in which the parasite count was 856 parasites per 100 fields the bilirubin was 1 0. units whereas in another with a parasite count of only 46 per 100 fields the serum bilirubin was . 0. units

Kingsbury s figures suggest that there may be some relation between the degree of bilirubinaemia and the size of the spleen in individual cases Other authors have not confirmed this (Wolski and Schewelewa 19.9 Schachsuwarly 19.7)

The figures published by workers in this field show a wide variety of quantitative results For instance Ross s figures for malignant tertian malaria are consistently higher than those of most other authors Ross accounts for this by suggesting that his high figures are the result of examining blood during quinine therapy In his series of 30 cases the five smallest concentrations were observed in untreated cases and most of the high concentrations were found in cases under treatment with either quinine or plasmoquin One untreated case had however a concentration as high as four units per 100 c c By closer observation of the bilirubin content of serum over a period of treatment in some of his cases he observed characteristic changes following quinine therapy In most patients the bilirubin rose noticeably during the first day of treatment but rapidly returned to normal after a few days This change is clearly illustrated in his case 3 in whom the serum bilirubin rose from 1 2 to . 0 units on the first day of quinine therapy and fell to 0 4 units by the third day Hughes and Shrivastava (1930) in several of their cases of chronic malaria with enlarged spleens also observed a rise in bilirubin within 20 minutes after intravenous injection of quinine

Fairley and Bromfield noted a fall to normal limits within a few days of both quinine and atebrin therapy neither of which alone affects the liver function and drew attention to the fact that in their cases the maximum concentration of bilirubin reached in the blood was independent of the degree of anaemia existing at the time of examination Thus in cases in which the mean red blood cell count was 4–5 million per cu mm the mean serum bilirubin measures 2 2 units and 1 8 units in benign tertian and malignant tertian cases respectively whereas in the group of cases with cell counts of 3–4 million per cu mm the mean serum bilirubin was 1 15 and 1 13 respectively for the two infections They pointed out that this discrepancy could be most easily explained by presuming that the quantity of bilirubin present at any one time was dependent on the current *rate* of destruction of red cells and not on the total number which had been destroyed On this assumption

the greatest degrees of bilirubinaemia might be expected to occur in blackwater fever where haemolysis is most rapid and severe This is usually the case With the exception of the figures of Yorke Murgatroyd and Owen the quantitative measurements of bilirubin observed by workers in blackwater fever have been very high indeed. For example Fairley and Bromfield recorded as much as 88 5 units in a fatal case These very high concentrations are usually observed during haemolysis but as Owen and Murgatroyd point out the excess bilirubin in blackwater fever may persist for some days after the haemolysis has apparently ceased These authors suggest that this continued increase in bilirubin may be the result of concomitant liver damage which causes slow excretion of the pigment and quote in support of their contention the experiments of Jones and Jones (1922) who showed that free haemoglobin disappears from the circulation in a matter of hours Fairley and Bromfield however refute this argument and consider the persistence of bilirubin in the blood is accounted for by its naturally slow excretion subsequent to the cessation of the haemolytic activity

Most authors regard the hyperbilirubinaemia of malaria and blackwater fever as the result of abnormally rapid blood destruction. Some however regard it as evidence of hepatic insufficiency especially in induced malaria For instance Wile and Sams (1934) state that although haemolysis may explain much of the mild jaundice with raised bilirubin seen in therapeutic malaria in syphilitics such lysis cannot account for the more severe icterus sometimes observed They consider that the serum bilirubin is seldom elevated sufficiently as the result of haemolysis to produce very obvious clinical jaundice and that the latter does not appear until appreciable hepatic damage exists Kopp and Solomon (1943) support this hypothesis as a result of the findings in one of their cases and consider that generalized jaundice (as distinct from mere scleral icterus) developing during malarial therapy derives from liver damage O Leary *et al* (1939) also take the view that jaundice associated with inoculated malaria is not entirely due to blood destruction and that there is a hepatic factor involved They suggest the unwieldy label haemohepatogenous for such forms of icterus

Differences of opinion are not surprising for although there is little doubt that the bulk of the bilirubin circulating in the blood derives from the products of haemolysis the degree of hyperbilirubinaemia attained must also to some extent be determined by its rate of excretion through the liver Physiological or anatomical derangement of

the latter organ may thus be involved to a variable extent in the production of hyperbilirubinaemia in malaria (Rich 1930) This derangement is indicated in much of the recent work in which changes in serum bilirubin content have been examined contemporaneously with liver function tests of one kind or another In the main the development of hyperbilirubinaemia has been found to run parallel with depression of liver function determined by such methods as laevulose tolerance hippuric acid synthesis and dye retention To this extent therefore it is reasonable to suggest that even though the production of excess bilirubin may itself be the direct result of excessive blood destruction the failure of its removal from the blood stream may be due to liver derangement

Fairley and Bromfield (1934) as we have seen apparently consider that the excretion of haemoglobin derivatives is normally a slow process so that hyperbilirubinaemia in the late stages of blackwater fever as reported by Owen and Murgatroyd may indicate neither hepatic involvement nor existing haemolysis Such a view is however not substantiated by the results of experiments in the treatment of polycythaemia vera with phenylhydrazin in which as a result of rapid blood destruction temporary embarrassment of excretion of the products of haemolysis is believed to occur and bilirubin accumulates in the serum with consequent production of icterus Huffman (1927) found that when jaundice developed in such cases the rise in bilirubin was only temporary and was not sustained and recovery was rapid and complete Other workers have also found the removal of the products of haemolysis is rapid in healthy animals Jones and Jones (19_2) for instance found the bilirubinaemia resulting from artificially induced haemoglobinuria in paroxysmal haemoglobinuria was of very short duration It should be noted that in the patient referred to by Owen and Murgatroyd excessive bilirubinaemia was present on the twenty-first day of the disease 19 days after haemoglobinuria had stopped and at a time when haemoglobin could no longer be detected in the plasma

From what has been said it is evident that the part played by the liver in the development and persistence of hyperbilirubinaemia in malaria is difficult to asses and on grounds of measurement of bilirubin alone probably cannot be estimated In view of the widely stated opinion that such hyperbilirubinaemia and associated jaundice is entirely haematogenous in origin it must be emphasized that so-called toxic liver damage can also give rise to an increase in the serum of a pigment indistinguishable from the bilirubin of haemolysis in

that it gives the indirect van den Bergh reaction and is not normally passed through the kidneys (Best and Taylor 1943)

When the direct van den Bergh reaction is also present it indicates some degree of difficulty in excretion of bile through the liver, especially when accompanied by the presence of bile pigments in the urine In naturally acquired malaria as has been noted such direct reactions are seldom observed Ross says they are never seen in uncomplicated cases *In* more severe malaria and in blackwater fever they are however reported not infrequently usually in association with clinical signs of liver involvement including bilirubinaemia and enlargement and tenderness of the organ There is little doubt that in these cases the liver has become actively involved, but it is not always easy to understand how such involvement occurs particularly in cases which ultimately recover normal liver function In extreme cases the liver at autopsy is found to be swollen and congested and there are often marked degenerative and necrotic changes in the liver cells and the biliary capillaries and gall bladder are often choked with viscid bile *Some degree of interference with excretion of bile in such* circumstances is not difficult to appreciate Changes like this are probably irreversible but in milder cases the changes are probably mainly functional with no obvious anatomical basis

In induced malaria the interpretation of all results must be modified by the clinical state of the patient since latent or active syphilis may assist the assault of malaria on the cells of the liver and give rise to changes which the disease would *not itself produce*

Fairley and Bromfield (1934) have drawn attention to the curious absence of severe bilirubinaemia in monkeys (*M mulatta*) infected with *P knowlesi* Although grave haemolytic anaemias develop rapidly in such animals the authors found no clinical evidence of jaundice and never more than 2 75 units of bilirubin in the plasma They note also that the plasma of normal *M mulatta* often appears to contain no bilirubin the van den Bergh reaction being frequently negative and suggest that the fate of haemoglobin in monkeys may not be the same as it is in humans

Bile pigments in urine in malaria and blackwater fever

It has been noted above that considerable jaundice may develop in malaria without the passage of bile pigments to the urine Kingsbury (1926) infers from this that obvious jaundice in the absence of bilirubinuria must be principally haematogenous

The failure of bilirubin in many cases of malaria to pass from the

plasma into the urine is in keeping with the fundamental physicochemical differences between the bilirubin resulting from biliary obstruction and that derived from haemolysis Hoover and Blankenhorn (1916) have shown that the former dialyses readily through a semipermeable membrane whereas the latter does not The absence of bilirubin in the urine in malarial jaundice is often considered to exclude the existence of any interference with the excretory function of the liver but it does not necessarily indicate that the excess pigment present in the serum is derived from haemolysis only because the bilirubin arising from toxic interference with liver cell function (e g in chloroform poisoning) is closely related to if not identical with that of haemolytic origin and is equally non-dialysable

When bilirubin passes in the normal manner into the intestine it is there reduced to urobilinogen and returned via the portal circulation to the liver The greater part is re-excreted into the intestine but some may escape into the urine and becomes oxidized to urobilin on exposure of the urine to air and light There is some evidence that the liver cells themselves may be capable of forming small amounts of urobilinogen and that the pigment so formed is thrown into the general circulation eventually passing into the urine but the bulk of the urobilinogen present in the body is apparently formed in the intestine and the liver functions mainly as a channel of its re-excretion into the bile (Elman and McMaster 1925)

Many authors regard the appearance of excessive urobilinogen and urobilin in the urine as evidence of liver damage (Wallace and Diamond 19_5 MacCormac and Dodds 19_3) According to Elman and McMaster however both pigments may be present after haemolysis when the excretory function of the liver is apparently normal in this case they result from the production in the reticuloendothelial system of more urobilinogen than the liver even with its great functional reserves can excrete

It is not surprising in view of what has been said that both urobilin and urobilinogen have frequently been found in urine in malaria and blackwater fever According to Owen (19_8) Grim was the first to demonstrate urobilinuria occurring during malarial fever This observation has been amply confirmed by many authors Simpson (1911) stated that a rise in urobilin occurs shortly after the onset of pyrexia in malaria and Reynolds (1919) found the concentration in the urine rose and fell roughly with the fever Gordon (19_4) observed an increase in the pigment especially during the acute phase of the attack of malignant tertian malaria Rin (1941) reported increases in

both urobilin and urobilinogen in some 60 per cent of acute cases and in many latent and chronic malignant tertian infections Sorensen (1914) found that in some of his cases the urinary concentration of urobilin rose sharply some hours before the onset of the paroxysm but this observation has not been confirmed

In view of the (then) uncertain chemical relation between urobilinogen and urobilin Kingsbury (1926) investigated the excretion of both pigments in malaria especially in malignant tertian He found that urobilin and urobilinogen concentrations in the urine increased during an acute malarial attack and that the concentrations reached were greatest in malignant tertian especially when there was jaundice Owen (1928) studied the excretion of urobilinogen thoroughly in 11 cases of benign tertian (six induced) 40 cases of malignant tertian (two induced) and four cases of quartan malaria (two induced) Excessive urobilinogen was found at times in the urine of most cases of naturally acquired malaria of all three types In the induced cases excess pigment was not recorded over the period of observation in quartan infection (two cases) and was found in only a few of the benign tertian infections (six cases) at irregular intervals Both cases of induced malignant tertian showed considerable concentration of pigment in the urine although one did not develop a heavy malarial infection Owen concluded that over long periods of observation 'excess of urobilinogen in the urine is by no means a constant finding in either simple tertian or quartan malaria and that over shorter periods excess may also be absent in infections with *P falciparum*

Greatest excess was present in Owen s cases in *P falciparum* infections This finding agrees with those of Simpson who found that the urobilin concentration was maximal in malignant tertian but contrasts with that of de Jonge (1904) who stated that the output of urobilin is highest in benign tertian. Gordon (1924) and Hehir (19-7) could find no significant difference between output of urobilin in the various infections

Owen found no relation between the fever and increased urinary excretion of urobilinogen. In many of his cases the patients suffered a series of severe paroxysms without any increase in urobilinogen output His findings agreed with those of de Jonge in that output of urobilinogen was increased by giving quinine Other observers have however noted a relation between urobilin and the presence of fever Thus Simpson says the output commences shortly after the onset of pyrexia and Reynolds that it rises and falls with the fever As men-

tioned above Sorensen stated that the amount of urobilin actually increased before the onset of the malaria attack

The excretion of both urobilin and urobilinogen has been watched by observers during induced malaria and in general the findings of Owen have been confirmed The output of the pigments is irregular and cannot be closely related to the severity or the clinical stage of the malaria attack Fredricks and Hoffbauer (1945) for example investigated the urinary excretion of urobilinogen in 24 syphilitic patients undergoing malarial therapy Significant increases were observed in 18 in eight of which there was some degree of hyperbilirubinaemia

Stephens and Christophers (1908) state that excretion of urobilin is common in blackwater fever usually at the time of haemoglobinuria or subsequent to it Sorensen (1914) held that the output of the pigment began to increase before the paroxysm and considered in view of its association with red cell destruction that its presence in high concentration might be a warning sign of oncoming blackwater fever He reported 16 cases in which high urinary urobilin concentration was regarded as suspicious of blackwater fever all developed blackwater fever within 10–20 hours of the observations Owen and Murgatroyd (1928) investigated the output of urobilinogen in two cases of blackwater fever over a period of 24 days and were unable to confirm Sorensen s observations They reported that th output in the urine gave no indication of the onset of haemoglobinuria being no higher before onset than in many cases of uncomplicated malaria The maximum concentrations of urobilinogen in one case were observed during the haemoglobinuric period with high values still present two to three days after the urine had cleared In the second case urobilinogen was present irregularly during haemoglobinuria and the concentration was greatest on the fourth day following temporary suppression of urine at a time when the urine was free from haemoglobin In a third case reported by these authors together with Yorke (1930) high values were recorded on the second day of haemoglobinuria and irregularly thereafter up to the ninth day five days after haemoglobinuria had stopped Urobilinogen was present on the day following the disappearance of haemoglobinuria and again five days later when a second wave of haemoglobinuria occurred (Yorke Murgatroyd and Owen 1930)

Thus in both malaria and blackwater fever urobilinogen and urobilin may be present in the urine They are not always present however during an attack of malaria and the quantities in the urine vary considerably in individual cases In blackwater fever urobilinogen is

normally in greatest concentration during or immediately after the passing of haemoglobin so that its presence appears to be related to the haemolytic process In malaria the relation to the haemolytic process is not so obvious

It is of interest to note here that So (1941) observed that increases in urobilin in malaria ran parallel to depression of the excretion of the pigment azorubin-S the latter finding being interpreted as evidence of hepatic dysfunction Other authors as we shall see have also observed that increases in urinary urobilin and urobilinogen coincide with deviations of so-called hepatic function-tests and may therefore be associated with hepatic insufficiency Some authors go so far as to consider the presence of excess of urobilin or urobilinogen as definite evidence of hepatic damage (Wallace and Diamond 1925 MacCormac and Dodds 1923 Fredricks and Hoffbauer 1945) In fact however assessment of liver damage on the estimate of the excretion of either urobilin or urobilinogen alone is not justified As is the case with all laboratory investigations of liver function the results of such single tests are significant only when considered with those of a series of others performed contemporaneously (White *et al* 1939 Higgins *et al* 1944)

Liver function tests in malaria

There have been relatively few attempts to assess the functional capacity of the liver during the course of naturally acquired malaria. Sinton and Hughes (1924) appear to be amongst the first to investigate the deviations of a specific liver function in malaria under controlled conditions They used a laevulose tolerance test in which the blood sugar curve was determined at regular intervals after the oral administration of 45 gm laevulose When the liver is functioning normally with regard to laevulose the blood sugar curve following such administration of laevulose does not rise more than 30 per cent above the fasting level and returns to within 15 mgm of normal within two hours Abnormality of function of the liver is indicated by elevation of the curve and by persistence of high levels after two hours According to McNee the laevulose tolerance test is positive in most cases of jaundice except those of haemolytic origin Using Tallermann's standards (1923) *Sinton and Hughes investigated 15 cases of acute P falciparum* infection which were not showing pernicious symptoms Some of these patients showed abnormal curves and the authors considered that in these there may have been some definite interference with the glycogenic function of the liver They could detect no relation between the

degree of rise in the blood sugar curve and the severity of the parasitaemia the size of liver or spleen or the intensity of jaundice which developed

Kern and Norris (1944) in their review of over 1 100 cases of all types of malaria reported enlarged livers in 59 out of 100 consecutive cases Some of these cases had hyperbilirubinaemia and showed retention of bromsulphthalein the latter indicating a derangement of the excretory functions of the liver

So (1941) observed the excretion rate of the dye azorubin-S in acute malaria and found that reduction of its excretion was associated with increased urinary excretion of urobilin This combination of reduced dye excretion and excessive excretion of urobilin was regarded as evidence of disturbance of liver function The changes were most pronounced during fever particularly in malignant tertian infections In these experiments the author could find no relation between the failure of dye excretion and the development of the Takata-Ara flocculation reaction which is dependent on the globulin content of the serum

Deviations in a similar test the cephalin cholesterol flocculation test were studied by Mirsky von Brecht and Williams (1944) in acute recurrent and chronic malaria In a detailed study of 10 patients infected with *P vivax* and *P falciparum* it was discovered that at some time or other during the disease the cephalin cholesterol flocculation test was positive In addition in most cases they found a high icterus index raised serum globulin and an increased erythrocyte sedimentation rate Some of the patients examined by Mirsky and his colleagues had been treated with quinine or mepacrine but the tests were done and found positive in some cases before these drugs were given so that they considered it unlikely that the reaction was affected by the drugs themselves On the contrary as the clinical condition improved under treatment the results of the flocculation tests returned to normal The authors considered their results indicated hepatic damage and suggested that this might be to some extent obviated by employing a more substantial diet during malaria fever They proposed a routine diet of high carbohydrates high proteins low fat and high vitamin content

McCorkle (1944) studied liver functions in 55 severe cases of malaria by means of the hippuric acid synthesis test which is designed to test the detoxicating functions of the liver The synthesis was normal in all except eight five of whom had scleral icterus and one of whom was appreciably jaundiced In six out of the eight patients exhibiting

reduced synthesis the test was normal by the twelfth day of treatment These patients were treated with quinine 2 gm daily for three days followed by mepacrine 300 mgm daily for a further five days and the synthesis tests were performed on the fifth or sixth days of treatment The author interpreted his results as indicating hepatic dysfunction so far as hippuric acid synthesis was concerned and considered that the antimalarial drugs did not affect the test since in his experiments neither quinine nor atebrin displayed any inhibitory action on liver function His results with regard to these drugs are in accord with those of recent work on the physiological effects of therapeutic and suppressive administration of mepacrine (*Lancet* 1945 Macgraith and Havard 1945 Army Malaria Research Unit 1945) and with those of other observers such as Kopp and Solomon (1943) who found that in the liver derangements of induced malaria recovery of function occurred during quinine therapy

Hepatic function tests have been employed also in patients suffering from therapeutically induced malaria Williams (1927) examined the oral laevulose tolerance test in a group of general paralytics and a tabetic who were given therapeutic malaria He used as controls general paralytics not receiving malaria and known cases of liver damage Laevulose blood curves were drawn before inoculation with malaria during the course of the induced malaria after recovery and during and after relapses when they occurred He found that in every case the tolerance curves observed towards the end of malarial therapy showed evidence of hepatic insufficiency In some patients the curves became abnormal after only one or two rigors In one case which died eight days after the malaria was terminated by quinine the deviation from normal was extreme and autopsy revealed acute yellow atrophy of the liver In most cases the curves returned to normal limits very rapidly after adequate treatment with quinine but the restoration to normal was slower in the more serious cases Hepatic insufficiency as displayed by deviation of the laevulose test was seen in four relapses Williams states that when they were measured the greatest changes in the indirect van den Bergh reaction *ran roughly parallel with the changes in the sugar curves* His results in his control groups are interesting In only one out of 13 general paralytics treated with arsenicals was there any disturbance of laevulose tolerance In seven general paralytics treated with relapsing fever there were no such hepatic effects

O Leary Greene and Rowntree (19_9) state that of 400 syphilitic patients treated with therapeutic malaria 6 per cent developed some

degree of jaundice Six of the latter showed bromsulphthalein retention The van den Bergh reactions in these cases have been referred to elsewhere in all the direct as well as the indirect reactions were positive There was enlargement of the liver in only one case in whom 11 days after onset of malaria the indirect van den Bergh reaction was 23.4 units and there was marked retention of bromsulphthalein on re-examination of the blood later when the patient had recovered the bilirubin was 0 6 units and there was no evidence of dye retention In all these six cases the bile acids the concentration of which is often increased in obstructive jaundice remained within normal limits but sometimes near the upper limits of normal during the active malarial infection O Leary et al in view of the fact that in their series of cases some degree of retention of dye and a direct van den Bergh reaction were constantly present considered that in therapeutic malaria in syphilitic patients there is always some involvement of the liver

Lippencott Ellerbrook et al (1945) carried out liver function tests in cases of chronic relapsing *P vivax* malaria They used the following tests bromsulphthalein retention cephalin-cholesterol flocculation galactose tolerance hippuric acid synthesis and measured in addition the serum bilirubin icteric index albumin globulin cholesterol and phosphatase and urinary urobilinogen The galactose tolerance tests were performed on patients 4 to 5 weeks after an attack of malaria and of 207 men so examined only seven showed abnormality in the excretion of the sugar Their results which were much the same as those of other workers indicated that transient derangement of liver function occurred in some cases but there was little or no evidence of permanent hepatic damage

Kopp (1942) observed progressive pronounced lowering of plasma albumin with varying changes in fibrinogen and globulin content at the onset of the paroxysm in induced malaria Fevers induced artificially by injections of typhoid vaccine or by inductothermy had no such effects He therefore considered that malaria infection interferes with the hepatic synthesis of albumin The increase in globulin could be explained by the increased haemolysis associated with the paroxysm The changes in fibrinogen observed by Kopp were equivocal but in subsequent work (Kopp and Solomon 1943) it was found that fibrinogen was definitely decreased during the active stages of induced malaria Radosalvjevic and Ristic (1926) report an increase in this substance at the height of the fever during a paroxysm This latter

observation is of course just the reverse of what might be expected in liver damage

Kopp and Solomon (1943) further investigated liver function in therapeutically induced benign tertian malaria (quotidian fever) in nine neurosyphilitic patients who were allowed to have 4 to 12 paroxysms before the administration of specific quinine therapy Six of these cases had not been previously treated for syphilis but three had had arsenical drugs In these studies the authors used a series of function tests including bromsulphthalein retention the cephalin-cholesterol flocculation and hippuric acid synthesis and the estimation of blood cholesterol (total free and ester) phospholipoids fibrinogen and total bilirubin

Before malaria therapy was begun eight of the nine patients gave normal values for all tests except four cases who showed a very small degree of dye retention and five in whom the concentration of fibrinogen was high The ninth patient had had arsenical hepatitis six years previously and showed considerable dye retention and poor hippuric acid synthesis During malarial therapy all cases exhibited signs of liver dysfunction determined by increased retention of dye reduced synthesis of hippuric acid positive cephalin-cholesterol flocculation reduction of fibrinogen concentration and a fall in total blood cholesterol especially in the ester fraction The authors considered this latter an indication of serious liver damage (White Deutsch and Maddock 1939) Phospholipoids were also reduced as they are in acute hepatitis Administration of specific drug treatment for malaria was followed in all cases by a return of the tests to normal values in three to six weeks apart from continued elevation of fibrinogen concentration in three cases Of the other tests the cephalin-cholesterol flocculation was slowest to return to normal The authors found that the degree of impairment of liver function did not depend on the number of malaria paroxysms suffered or the total duration of active malaria

Kopp and Solomon concluded from their observations that therapeutic malaria gives rise to disturbance in liver function of a transient nature The findings in one patient who became severely jaundiced during malaria therapy indicated that jaundice of malaria may be due to liver damage They do not consider that the rapidly developing haemolytic anaemia of malaria in itself affects the liver function since they quote Deutsch as stating that intravascular haemolysis of red blood cells such as is produced by autohaemolysins does not result in abnormal liver function tests nor is liver function impaired by secondary anaemia itself In this they are supported by the findings

of Huffman in his studies of the effects of phenylhydrazine in polycythemia (1927) They point out however that the reduction in blood protein observed in malaria may be a factor in promoting liver insufficiency since it has been shown that impairment of liver function develops readily in protein depleted animals (Elman and Heifetz 19 0 Kopp and Solomon 1941)

Fredricks and Hoffbauer (1945) recently made a careful survey of hepatic function in induced malaria Their research was started as the result of the death subsequent to malaria therapy of a neurosyphilitic patient in whom there was found an acute degeneration of the liver and portal cirrhosis These authors gave benign tertian malaria to 20 male and 11 female syphilitic patients who were allowed 8 to 10 paroxysms each The cases were all neurosyphilitics and none had manifest liver disease before treatment The first 20 patients were given the ordinary hospital diet but the last 11 in view of the consistent evidence of hepatic involvement of the others were given alternately the ordinary diet or a liver sparing diet consisting of high carbohydrate high protein and low fat Clinical signs of liver involvement described as purpura scleral icterus and nodular enlargement of the liver appeared in one case after eight paroxysms Enlargement of the liver was noted in three The changes in total bilirubin and direct van den Bergh reaction observed by these authors have already been described in the section on bilirubinaemia In addition to these increases in bilirubin 24 patients developed significantly increased cephalin-cholesterol flocculations The urinary urobilinogen was excessive in 18 Five out of eight cases examined before during and after malaria therapy showed maximal dye retention during the malaria Diet apparently made no difference to the appearance of deviations in the tests

The authors summarize their findings by stating that all patients adequately studied showed some evidence of hepatic dysfunction

Glenn *et al* (1946) have recently reported their findings in a series of liver function tests carried out on a group of 60 neurosyphilitics who had been receiving mapharsen and bismuth treatment for some time prior to the induction of *P vivax* and *P malariae* malaria The blood bilirubin was raised in some There was reduction in hippuric acid synthesis and the total plasma protein fell during the febrile period with the usual changes in the albumin/globulin ratio The hippuric acid synthesis and the plasma protein concentrations were restored to normal soon after termination of the malaria with atebrin and mapharsen but the cephalin-cholesterol flocculation reaction returned only slowly to normal a finding similar to that of Kopp and

Solomon (1943) The authors consider that their results indicate temporary hepatic dysfunction during the malarial therapy particularly in respect of the hepatic detoxicating powers

Similar evidence of hepatic insufficiency has also been noted by Lippencott *et al* (1946) in induced *P vivax* malaria in neurosyphilitics In some of their cases tests conducted after the termination of the malaria showed restoration of hepatic function to the level present before therapy

Carter and McLagan (1946) have recently investigated the results of two tests of liver function (the serum colloidal-gold test and the thymol turbidity test) in a variety of conditions including congestive cardiac failure glandular fever and *P vivax* malaria and report that in the latter disease all except two of 35 patients gave a positive colloidal-gold reaction and 28 gave a positive thymol turbidity reaction In *seven cases the tests were repeated and found normal* after three weeks treatment Urobilin was found in the urine in 20 out of 21 cases examined The authors point out that these flocculation tests probably depend principally on changes in serum gamma globulin Increases in gamma globulin occur in liver disease but are not confined to such being also found for example in rheumatic fever aplastic anaemia and peritonitis It is possible also that circulating antibodies may be found within the gamma globulin fraction (Enders 1944) They consider that liver dysfunction is indicated by the presence of urobilin in the urine in malaria coupled with the results of the flocculation tests The formation of antibodies may however partly contribute towards the latter reactions and interference with the hepatic blood supply may be concerned in the hepatic dysfunction

The investigations that have been outlined above show clearly that in many patients during the acute phase of both naturally acquired and artificially induced malaria deviations in the standard liver function tests can be demonstrated The assessment of such results in terms of liver damage depends on the credence that can be placed on the tests used Examination of one test alone such as the use of the laevulose tolerance test by Sinton and Hughes in naturally acquired malaria and by Williams in artificially induced malaria is probably not sufficient but the value of repeated serial tests as measurement of liver function is now widely accepted (Higgins *et al* 1944 Best and Taylor 1943 White *et al* 1939) It appears therefore that malaria infection can bring about inhibition of certain liver functions even in the absence of the usual clinical signs of hepatic insufficiency Such disturbances are of a transitory character and except in cases where

the liver is grossly damaged restoration of normal liver function takes place rapidly after the termination of the malaria attack After a single attack little in the way of permanent damage occurs but although there appears to be no work clearly defining the effects of long continued low grade or chronic malarial infection there is some evidence to suggest that permanent liver damage may ultimately result The means whereby the liver cells become damaged in malaria will be discussed later

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For instance in acute severe *P knowlesi* infections in monkeys there may be little structural alteration in the liver but the glycogen content of the polygonal cells is often appreciably lowered (Fulton 1939)

When the severity of the infection or its complications reaches a certain point the changes produced in the cells may be irreversible and structural alterations become visible Such changes in the cells result directly from the malaria infection or from tissue reaction to the invasion or indirectly from changes in blood flow through the liver and associated anoxic conditions of the cells Many workers have suggested that some soluble chemical substance either in the form of a so-called toxin or a metabolite such as pyruvic or lactic acid may be generated by the parasite and be vitally concerned in the pathogenesis of liver damage Others believe that the products of schizogony released into the general circulation during the paroxysm may be concerned

Macroscopic appearances

The liver in acute malaria is often uniformly enlarged due partly to congestion and partly to swelling of the hepatic polygonal cells plus hypertrophy and hyperplasia of the reticuloendothelial cells Such enlargement has been reported in *P vivax* and *P malariae* as well as in *P falciparum* infections Occasionally in some very severe and rapidly fatal infections the liver may be reduced in size

According to Gaskell and Millar the size of the organ in malignant tertian malaria depends to some extent on the clinical type of the infection. Thus in the group I cases of these authors (the so-called true cerebral type) in which the parasites were chiefly concentrated in the brain the liver was large smooth and firm in group II (the septicaemic type) where there was intense proliferation of parasites in the blood stream the liver was generally large and soft in group III (the cardiac type) in which there was a history of repeated attacks of malignant tertian the size of the organ varied In the view of these authors the size of the liver was apparently mainly dependent on the amount of congestion this in itself being related to the condition of the heart Many other workers refer to the variation in the degree of hepatic congestion observed in malaria but most agree that some congestion is usually present Klotz for instance noted that in his autopsies of children under ten the liver was intensely congested such congestion was less pronounced in the more necrotic livers

Taliaferro and Mulligan (1937) state that the changes in size of the liver in monkey and bird malaria are much the same as those seen in

CHAPTER VI

THE LIVER IN MALARIA AND BLACKWATER FEVER

(ii) *Pathology and Pathogenesis*

PATHOLOGY Macroscopic appearances — Histological changes General picture Malaria parasites Pigment The blood vessels Changes in the liver cells Regeneration cellular infiltration and repair Liver changes in monkey malaria PATHOGENESIS Right heart failure — Congestion without heart failure Chiari's disease — Acute obstruction to venous flow — Changes in the vessels giving rise to vascular obstruction — Hepatic anoxia — Antigen antibody reaction — Nutritional status — *Syphilis antimalarial drugs* RECAPITULATION *Liver lesions* in malaria and blackwater fever as examples of an hepatic syndrome of wide distribution

PATHOLOGY

IN human malaria few cases other than severe *P falciparum* or blackwater fever ever reach autopsy so that the pathological picture generally described represents the findings in only the most serious forms of the disease There is little information about the anatomical appearance of the liver in the uncomplicated acute attack and except in a few human cases in which biopsy of the liver has been performed most of our knowledge has been derived by analogy from investigation of animal malaria particularly *P knowlesi* and *P cynomolgi* infections in rhesus monkeys It may be said in general that in the uncomplicated case the deviations observed in liver function tests are not reflected in any appreciable changes in the macroscopic and microscopic appearances of the liver Fredricks and Hoffbauer (1945) for instance describe a case in which *liver function tests were normal* before the administration of therapeutic malaria and became abnormal during the attack so that the cephalin-cholesterol flocculation became positive some retention of bromsulphthalein was observed and urobilinogen was excreted in the urine in excess although the serum bilirubin was not increased Liver biopsies before and after therapy showed no serious hepatic changes

Thus little anatomical evidence of change in the liver can usually be found associated with even gross deviations of function tests probably because the latter often represent reversible changes of function and so are not visibly reflected in the liver substance itself Sometimes however evidence of liver derangement can be obtained in other ways

changes are usually limited to the central zone of the lobule Where necrosis is pronounced the necrotic areas are yellowish grey and contrast clearly with the brownish-red background of the rest of the liver substance Klotz in a series of severe malignant tertian infections in West Africa found that the individual lobules were plainly marked out by the presence of yellow-grey centres of degeneration and necrosis so that the freshly cut surface gave the appearance of reversed nutmeg detail

Malarial pigment is scattered more or less evenly through the liver substance in the acute stages of malaria its distribution as Taliaferro and Mulligan point out being determined by generalized phagocytosis by reticuloendothelial cells In the acute disease there may be so many parasites and so much pigment circulating that all the reticuloendothelial cells lining the sinusoids are presented with equal opportunities of phagocytosis In malaria of long standing however the malarial pigment is found predominantly in cells near the periphery of the lobule a fact probably explained by the entry of parasitized blood from the portal system at the periphery According to Taliaferro and Mulligan in chronic malaria the relatively few circulating parasites and the associated pigment are phagocytosed by the reticuloendothelial cells at the periphery and become filtered out of the circulation before the blood reaches the middle and central regions of the lobules

The amount of malarial pigment present may be sometimes so great that the liver is slate grey or even black in colour Such deep pigmentation is most commonly seen in malignant tertian infections especially according to Gaskell and Millar in subjects in whom there has been a general extensive invasion of the red cells throughout the circulating blood

The consistence of the liver varies with the type of infection the degree of congestion and amount of necrosis and other cellular changes such as regeneration As a rule in acute malaria it is softer than normal and may be friable especially when there is necrotic tissue present Some workers have described an increase in fibrous tissue in chronic and long sustained malaria and have referred to malarial cirrhosis The existence of such cirrhosis has however according to Taliaferro and Mulligan never been securely established

The cut surface of the liver in acute malaria is moist and bleeds easily especially from the congested and often dilated lobular veins In severe cases there may occasionally be scattered small haemorrhages These are however uncommon (Craig Dudgeon and Clarke etc) The

human although occasionally extreme hepatomegaly may occur in avian malaria particularly the chronic type Macroscopic evidence of fatty degeneration was not observed in monkeys by these authors

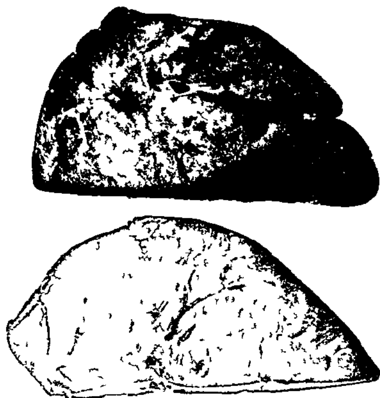


Fig 4—Pigmentation of liver in a case of recurrent malignant tertian malaria (upper section) Section of normal liver below

The colour of the malarial liver depends upon the state of congestion the amount of pigment taken up by the reticuloendothelial cells and the presence or absence of fatty degeneration or necrosis of the liver cells

In the acute case the liver is usually dark chocolate red and bleeds readily on section It may be reddish-yellow or streaked with yellow and appear greasy on cutting if fatty changes are pronounced Fatty

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cut edges may evert when there are early degenerative changes with consequent swelling in the polygonal cells

Not infrequently especially in blackwater fever the gall bladder at autopsy is found distended with thick tarry viscid inspissated bile which may be almost solid The possibility that such inspissation of the bile may cause secondary effects by obstructing the flow of bile and so account for the direct van den Bergh reactions sometimes recorded has been pointed out by Ross (1927) and Owen and Murgatroyd (1928) Fairley and Bromfield found in two fatal cases of blackwater fever that the gall bladder was distended with thick black tarry bile the excessive viscosity of which must have made its periodic discharge into the duodenum difficult They investigated the bilirubin content of this thickened bile and found it contained five to seven times as much as normal bile They point out the similarity of their results with those of Barratt and Yorke (1914) who showed that following the production of a high degree of haemoglobinaemia in rabbits the bile pigment content of the bile fluid did not increase by more than four to six-fold The increased viscosity of the bile in fatal blackwater and malaria cases is *probably related to the general dehydration common* in those conditions

Histological changes

General picture

The lobular structure is indistinct When the organ is congested the central vein and its tributary sinuses and sinusoids are filled with red cells many containing malaria parasites When anaemia is severe however the central vein and sinuses appear dilated and empty In most cases there is very little evidence of thrombosis or stasis in the smaller blood vessels and sinuses Malarial pigment abounds in the cells lining the sinusoids These cells are hypertrophied and often hyperplastic and contain actively phagocytosed red cells both parasitized and unparasitized as well as malarial and blood pigments They may be greatly swollen and project into the lumen of the vessel sometimes apparently blocking it The liver cells do not contain malarial pigment but may show granules of haemosiderin derived directly from breakdown of haemoglobin pigments The columns of cells are often widely separated partly because of dilatation of the sinusoids or swelling of the littoral phagocytic cells and partly because of atrophy of the hepatic cells themselves All stages of degeneration from the mildest form of granular degeneration to fatty degeneration

and the most severe necrosis may be found Such necrosis when clearly evidenced most frequently occupies the central region of the lobules involving especially the cells immediately surrounding the central vein The liver cells near the portal systems take staining better than those in the central zone and even when necrosis is advanced are usually relatively healthy in appearance in comparison with the more centrally placed cells Some degree of cellular infiltration may be present usually most concentrated in the vicinity of the portal vessels Occasionally small interstitial haemorrhages occur in relation to the central vein of the lobule Such haemorrhages are seldom extensive In severe malaria and in blackwater fever the bile canaliculi and sometimes the bile ducts may appear choked with thick bile

Malaria parasites

Parasites in various stages of development are usually easy to find in histological sections and smears of the liver after death from malaria They usually occur within red cells the latter being either free or phagocytosed by macrophages but sometimes may be found in the latter free from erythrocytes The number present varies with the type of clinical attack Gaskell and Millar for instance found there were few parasites in the liver in cases of malignant tertian in which the emphasis of the attack was on the brain whereas they were plentiful in cases in which there was a generalized parasitaemia These workers and Dudgeon and Clarke agree that the degree of invasion of red cells in the liver is relatively less intense than in the spleen Klotz found parasites equally readily in the sinuses of both children and adults dead from malignant tertian malaria Marchiafava and Bignami (1894) and Thayer (1899) state that they are more numerous in the portal and capillary vessels than in the lobular and hepatic veins

Pigment

Malarial pigment is not seen in the polygonal liver cells It is found only in the cells of the reticuloendothelial system Taliaferro and Mulligan as a result of much careful experiment in monkeys and birds state in agreement with most workers that the cells chiefly concerned are those lining the sinusoids the so-called Kupffer cells The undifferentiated endothelial cells lining the sinuses are not phagocytic *according to these authors but may be differentiated under the* stress of malaria into actively phagocytic monocytes indistinguishable from the Kupffer cells The endothelial lining of the smaller blood

vessels is not phagocytic and does not therefore contain malarial pigment Phagocytes from other tissues may find their way to the liver via the circulation and may contain pigment Some of these may come from the spleen via the splenic vein In support of this it has been claimed by Le Dantec (1925) that the blood in the portal vein (i e entering the liver) contains a higher proportion of macrophages than that in the hepatic vein (i e leaving the liver) Some authors have reported the presence of pigmented polymorphs in the vessels especially in chronic malaria

Malarial pigment occurs both in association with parasites and independent of them The phagocytes may contain at the same time *parasitized red cells unparasitized red cells and pigments during* the acute stages of the disease but in the latent or chronic infection the phagocytes contain only pigment which disappears from them extremely slowly The amount of pigment present its colour and the shape of its granules vary with the type of malaria and the clinical history of the case As has been said above pigmented phagocytes are to be found throughout the liver lining the sinusoids and sinuses in all parts of the lobule in severe acute malaria whereas in chronic malaria the pigmented phagocytes are most frequently found concentrated towards the periphery of the lobule The pigment as it occurs within the Kupffer cells has been described *variously by different authors* but it occurs most commonly in the form of large or small granules and coarse thick masses or balls the former being the commonest in acute and the latter in chronic malaria infection

Extracellular malarial pigment has been reported by some authors lying free in the sinuses (Dudgeon and Clarke 1917)

In addition to malarial pigment the phagocytic cells of the liver may also contain granules of haemosiderin This pigment is commonly present in the hepatic cells in which it may appear in considerable amounts especially in chronic or severely haemolytic malaria Dudgeon and Clarke (1919) *state that the ferrocyanide* reaction for free iron which is given by haemosiderin is absent or only slightly present in acute malaria It is more pronounced in chronic long-standing cases of malaria Klotz reports a small amount of free iron in the liver cells in his acute cases According to Taliaferro and Mulligan it is found in greatest concentration in the region of the central vein

The blood vessels

The state of the blood vessels capillaries and sinusoidal vessels of the liver substance and the efficiency of the blood flow through

them are matters of very great importance in the pathogenesis of the degenerative and necrotic changes seen in the organ in severe malaria and blackwater fever

Evidence of damage to the endothelial cells lining the vessel walls has been recorded by some authors There has however been a good deal of confusion over nomenclature and the term endothelium has been frequently used to include not only the cells lining the blood vessels but often most of the macrophages both fixed in the tissues and free It is necessary here as Taliaferro and Mulligan point out to distinguish clearly between the relatively non phagocytic true endothelial cells lining the ordinary blood vessels and capillaries and the specialized actively phagocytic cells lining the sinusoidal vessels The former the true vascular endothelium may sometimes be swollen in malaria and show mild granular or fatty degenerative changes and occasionally even necrosis Some degree of phagocytic activity has often been attributed to these cells but it is probable that in most cases this is the result of confusion between them and the reticular cells lining the sinusoids In general the pathological changes visible in the true endothelial cells in the liver are considerably fewer and less severe than those found elsewhere e g in the spleen or the brain but occasionally they may be severe For instance Gaskell and Millar (1920) observed in many of their malignant tertian cases that the endothelial cells of the liver capillaries were often swollen and fatty the nuclei in some being faintly stained these changes were particularly noticeable in the septicaemic type of the disease

Taliaferro and Mulligan describe two types of cells lining the liver sinusoids These are (i) the actively phagocytic swollen reticular cells usually called Kupffer cells and (ii) undifferentiated cells resembling the endothelium of ordinary blood vessels but which are distinct from the latter in being potentially capable of developing into phagocytic cells undistinguishable from the Kupffer cells According to these workers the littoral cells of the sinusoids are either phagocytic or capable of developing phagocytic activity under such stresses as malarial infections Phagocytic activity is accompanied by both hypertrophy and hyperplasia of the cells concerned so that the sinusoids may not infrequently appear completely obliterated by swollen phagocytes This phenomenon was recorded many years ago by Guarnieri (1886) and by Marchiafava and Bignami (1894) and has frequently been observed since The cells concerned usually show signs of intense phagocytic activity and may contain parasitized and unparasitized erythrocytes remnants of free parasites and granules and masses of

malarial pigment The cytoplasm may be so greatly swollen that it is sometimes difficult to distinguish the cells from the polygonal hepatic cells a circumstance which probably explains the claims of some authors that under certain conditions the true hepatic cells may be phagocytic (A full account of the phagocytic powers of the various forms of liver cells is given in an excellent review by Taliaferro and Mulligan (1937) of the histopathology of malaria) According to some observers the swelling of the littoral cells may distort the arrangement of the columns of polygonal hepatic cells sometimes pushing them widely apart Thus Gaskell and Millar describe wide spaces between rows of shrunken cells filled with swollen stellate and endothelial cells Opinion is divided about whether such phagocytes become seriously damaged in malaria and blackwater fever but various changes have been described in the heavily pigmented macrophages of the malarial liver which most probably must be considered identical with the littoral cells of the sinusoids Thus granular and fatty degeneration of these cells (which may be free in the lumen of the vessels) and occasionally necrosis with loss of nuclear staining have been described (Gaskell and Millar 19_0) On the other hand some authors state that in their experience serious degenerative and necrotic changes are confined exclusively to the hepatic polygonal cells (Klotz 19_9)

It is evident from what has been said that the changes found in the true endothelium are unlikely to disturb the flow of blood through the vessels whereas the passage of blood through the sinusoids which are normally tortuous and irregular in diameter may be impeded to some extent by partial obstruction or occlusion of the lumen by swollen littoral cells The blood flow may be deranged in other ways In the first place there is frequently a general congestion of the organ In some instances this may arise from raised hepatic venous pressure caused by a failing heart (e g in the septicaemic type of infection when the heart may be greatly dilated Gaskell and Millar) In others as we shall see later there is probably some kind of active obstruction to the escape of blood from the hepatic venous system to the vena cava In either case the result is congestion and dilatation of the central lobular veins which can be clearly seen in histological sections The tributary sinusoids are usually involved particularly towards the centre of the lobule and according to Gaskell and Millar in the region just under the capsule of the organ In extreme cases the whole vascular system of the lobules is grossly distended Where there has been severe or long continued haemolysis and a consequently low red blood cell

count the vessels may appear distended or patent and contain relatively few corpuscles [1]

The smaller vessels sinusoids and even the sublobular veins have sometimes been reported obstructed or partly obstructed by collections of macrophages usually heavily pigmented and actively phagocytic or of parasitized red cells which in the larger vessels may tend to accumulate near the endothelial lining (Guarnieri quoted by Marchiafava and Bignami 1894 Dudgeon and Clarke 1917) Large numbers of cast off fatty liver cells and pigmented macrophages have also been seen in the larger hepatic veins (Gaskell and Millar)

Thrombosis stasis and agglutination of red cells parasitized and unparasitized in the small blood vessels and sinusoids have all been described and some authors have suggested that the areas of necrosis observed in the parenchyma are usually associated with such obstruction to the vessels Dudgeon and Clarke (1917) refer to such thrombosis in some of their cases of malignant tertian infection in troops in Macedonia and state that where thrombosis occurs the component red cells are heavily parasitized Dudgeon (19_0) further states that in some cases the sinuses contain agglutinated erythrocytes The existence of any appreciable degree of thrombosis is vigorously denied however by many workers and the concensus of opinion seems to be that vascular thrombosis in the liver is rare in malaria Klotz states that in his series of cases of young children who had died of malignant tertian malaria there were no thrombi composed of erythrocytes parasitized or unparasitized or of leucocytes in the sinuses He found in adults that the central vein of the lobule and its tributaries were patent and congested with blood cells and showed no evidence whatever of thrombosis or other form of obstruction He states that the picture in the liver as far as the small vessels are concerned is nothing like the brain

The vessels of the stroma of the liver are not usually congested or otherwise affected in malaria except for the appearance in the portal regions of perivascular accumulations of round cells which will be discussed later Marchiafava and Bignami state that parasitized red cells are more prevalent in the portal vessels and capillary network than in the hepatic veins This may possibly be explained by the

[1] S th h p w wr en Hi worth (*Lectu s* *tl L er d t D e* Bl kw ll 1947) h d w t t the p t of well g f h p en hyn al ll in th proda t of trıl b l c in bo tr chl d poison g In hi w this w ll g lead t un fetet w th ntr lobul blood fl w th o gh oblit atio of the si uso d a d so to cent ilobular d gen n and necro is W h v co firm d Himsworth s observation n c bo t trachl rid po ni g b t h not been bl to find idence t how that m l e swell g f the p lyg nal c lls i pr minent n mala l infectio s

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the latter case to be certain whether the changes observed may not in fact be due to post mortem degeneration rather than to the malarial infection

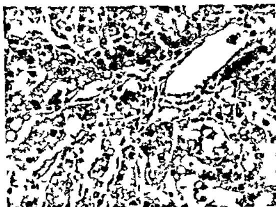


Fig 6—Fatty degeneration of polygonal cells of the liver in malignant tertian malaria

Fatty degeneration is common The fat occurs either as evenly distributed fine dots or as globules often of considerable size and sometimes occupying most of the cytoplasm In some livers the total fat is clearly increased but in others especially those of patients dying from very acute malaria or blackwater fever it is probable that the fatty change is more in the nature of a phanerosis Fatty degeneration is seen most commonly in the cells in the central zone of the lobule but where these are necrotic fat may be present in the cells at the periphery of the lobule frequently associated with granular cytoplasmic changes and sometimes with some loss of nuclear staining Dudgeon and Clarke noted fatty degeneration of one kind or another in about a third of their cases of malignant tertian malaria Most degeneration was observed in a case of blackwater fever in which the fatty changes were practically limited to the central zones of the lobules Gaskell and Millar (1920) describe the appearance of fatty globules in all their malignant tertian cases In the cases of cerebral malaria the most degenerate cells were those near the central lobular vein and here the fatty content was often excessive and there were often globules of large size In cases in which the parasitaemia was severe most of the liver cells were affected and the cytoplasm especially in the central zones was infiltrated with small fat droplets In the third type of case in which the cardiovascular system was mainly involved the liver

phagocytosis of parasitized cells while the blood is passing from the portal vessels through the sinusoids to the central vein (Taliaferro and Mulligan 1937)



Fig 5—Atrophy and necrosis of liver cells in n align nt tertain malaria Note central distribution of les ons and relatively normal appearance of peripherally placed cells

Haemorrhages into the substance of the liver have been reported in both malaria and blackwater fever They are infrequent and may occur in any part of the organ but are commonest in the region of the central veins of the lobules They have occasionally been described as subcapsular (Thomson 19_4)

The tissues of the liver may rarely show histological evidence of oedema presumably developed as a result of changes in the permeability of the small vessels Such oedema with separation of the cell columns has been described by Thomson in blackwater fever

Changes in the liver cells

Biopsy of the liver in acute uncomplicated malaria usually reveals no obvious anatomical changes in the cells but in fatal cases and especially in blackwater fever degenerative changes are common and may appear in all stages from simple granular degeneration of the cytoplasm to frank necrosis with nuclear involvement Cloudy swelling with granular cytoplasm and apparently healthy nuclei has often been reported sometimes accompanied by vacuolation Such slight changes are usually found in association with more severe degeneration but occasionally occur independently It is difficult in

to the necrotic areas As mentioned above Klotz described the appearance as one of reversed nutmeg details —a peripheral red zone



Fi 7—Atroph y and os of l cell in bl ckw ter F er N t the similarity d trib n of the p thological ha ges w h those n m l gr nt tertia malaria (F o 5)

and yellow-grey centre Histological examination revealed necrosis which in most cases occupied the inner two thirds of the lobule and was most marked in the cells surrounding the central vein Here the cytoplasm was granular vacuolated sometimes fatty and markedly acidophilic The nuclei stained badly or were pyknotic broken up or absent The area of necrosis in the lobule was sharply limited and the cells at the periphery in the region of the portal systems were relatively unaffected except for fatty changes in the cytoplasm (see also Dudgeon and Clarke 1917 1919 Gaskell and Millar) Klotz considered that the dilation of the central veins and sinusoids (described in the previous section) was a significant liver lesion and developed before the changes in the hepatic cells

In many cases of blackwater fever and in some of fatal malaria the bile found in the gall bladder is thick tarry and inspissated and appears capable of causing obstruction to the flow of bile from the gall bladder to the intestine and hence from the liver to the gall bladder Associated with these changes especially in the presence of severe necrosis the liver cells may show signs of biliary obstruction It is not uncommon to find the canaliculi choked and distend d with dark greenish bile in such cases especially in the central region of the lobule The smaller

cells were not greatly damaged but those at the periphery of the lobules contained globules of fat and those in the central zone showed all stages of fatty degeneration sometimes associated with poorly stained nuclei Klotz (1929) has also described fatty changes in the liver cells in malignant tertian malaria In his series of autopsies on children under the age of ten he found acute parenchymatous degeneration *with little evidence of fat but in older children there was a variable* amount of fatty change in the form of discrete globules in the cytoplasm of the cells particularly in the portal zones Occasionally the central zone was entirely occupied by a fatty degeneration of liver cells and in some cases such fatty degeneration was the only lesion present Similar changes have been observed by other workers in both human and monkey malaria (Rigdon and Stratman-Thomas 194- Kean and Smith 1944 Taliaferro and Cannon 1936 Taliaferro and Mulligan 1937 Napier 1946)

The ultimate stages of degeneration i e the irreversible changes of necrosis involving both cytoplasm and nuclei of the liver cells are frequent in fatal severe malaria and blackwater fever Such changes probably also occur in some non-fatal cases and the tissue concerned is ultimately replaced by processes of regeneration or fibrosis In human malaria particularly malignant tertian the latter changes are obscured by the degenerative processes In the most severe cases of liver damage practically all the cells show some degeneration or necrosis but more frequently the necrotic areas are scattered irregularly through the organ The most severe changes are sometimes seen in the immediately subcapsular tissue The areas of necrosis may appear as necrotic foci *which according to Thayer (1899) resemble those seen in typhoid* fever or the toxic foci of diphtheria and other infections (Dudgeon and Clarke 1917 Taliaferro and Mulligan 1937) Rigdon and Stratman-Thomas (194-) however point out that in malaria the necrotic cells are nearly always confined to the central zone of the lobule and cannot therefore be regarded as similar to necrotic foci seen in other infections which may appear in any part of the lobule Such centrally placed necrosis has been described over and over again in both malaria and blackwater fever and must be considered one of the most significant and characteristic features of the pathological findings There is no point in cataloguing accounts of such central necrosis but space may be given to Klotz s (1929) description of it in West African malignant tertian malaria On examination of the cut surface of the liver the lobules were poorly defined but the individual lobules could be picked out because of the presence of yellowish-grey centres corresponding

malaria one of the best descriptions being that of Marchiafava and Bignami (1900) who earlier (1894) reported signs of fresh cellular formation in malarial livers It is perhaps worth while quoting their account which refers solely to chronic malaria

The most important condition in this stage is the beginning of the new formation of hepatic cells This process usually begins towards the middle of a lobule whence it extends throughout a large part or even the whole of the lobule The newly formed hepatic cells are arranged as cellular cords by the side of the remnants of the necrosed hepatic cells around which are accumulated multinuclear lymphocytes some containing pigment others clinging to the necrotic detritus in such a fashion as to suggest the idea of a phagocytic function destined to carry away this detritus which would then be replaced by the young hepatic cells

Repair in the sense of replacement of damaged and necrosed tissue by fibrous tissue has been described by some authors Such fibrosis is complicated by the processes of regeneration referred to above and as far as can be judged from what little evidence is available is seldom extensive Hyperplasia of the perilobular connective tissue has also been reported but never appears extensive Marchiafava and Bignami considered that such increase in fibrous tissue was not a true cirrhosis and this view has on the whole received general support

Infiltration of Glisson s capsule has been described frequently especially in the periportal tissue Gaskell and Millar for instance found some degree of round cell infiltration of the capsule in their malignant tertian cases and stated that the accumulations of cells were most concentrated about the branches of the portal vein and hepatic artery but in some cases were generally distributed through the capsule Such infiltration was most marked in those cases in which the clinical signs were mainly cerebral The cells tended not to invade the peripheral regions of the lobules except where the cases showed signs of cardiac failure

Taliaferro and Mulligan demonstrated similar round cell infiltrations concentrated about the vessels of Glisson s capsule in monkey malaria and refer to such accumulations as mantling In *P knowlesi* infections of *M mulatta* the degree of mantling observed depended on the type of clinical attack Thus in the late stages of acute untreated infections no changes were observed in Glisson s capsule but some mantling appeared in two out of four monkeys in which fatal relapses supervened after apparent cure of the primary attack The monkeys in the livers of which mantling of the vessels was found had both been infected with

bile ducts are also frequently found distended with apparently viscid bile (Whipple 1909 Dudgeon 1920 Paterni 1923 Marchiafava and Bignami 1900 Rapoport 1928 Thomson 1924) Sometimes however the bile appears normal and flows easily In such cases there is little evidence of biliary obstruction and the liver cells are apparently free from bile even when there is considerable necrosis (Klotz 1929 Gaskell and Millar 1920)

Amyloid degeneration of the liver cells in chronic malaria has been described (Marchiafava and Bignami 1900)

In addition to degenerative changes some degree of atrophy of the liver cells is commonly seen in malaria associated as a rule with wide separation of the columns of cells Gaskell and Millar for instance describe rows of shrunken cells in the central zone of the lobule in cases of cerebral malaria and state that in cases with a high degree of parasitaemia the liver cells were shrunken and degenerate and the cell columns widely separated especially at the centre of the lobule Similar atrophy was described by Marchiafava and Bignami (1894) The atrophic cells are characterized by reduction of cytoplasm which may be fragmented and distorted and changes in the nuclei which may fail to take stains normally

Regeneration cellular infiltration and repair

In acute human malaria active tissue response to the invasion is usually secondary to the processes of destruction so that signs of regeneration and repair are not so well marked as those of degeneration and necrosis Nevertheless increased mitosis and division of both reticular cells and the polygonal hepatic cells have been described The multiplication of the reticular cells follows the intense phagocytosis of red cells parasitized red cells and pigment which takes place in the sinusoids and is thus in the nature of a direct response to the disease processes Division and increase in numbers of the hepatic cells should be classified more as an attempt at repair and replacement of damaged tissue Such multiplication of the polygonal cells has been recorded in both acute and chronic human malaria (Marchiafava and Bignami 1894 1900 Deaderick 1911 Thayer 1899 Craig 1909 etc) Gaskell and Millar (1920) observed changes suggestive of regeneration in the livers of some of their cases who had died of cerebral malaria These changes were found amongst the less degenerate cells in the peripheral regions of the lobules Here some cells were large and had exceptionally large nuclei others had two nuclei Regeneration has been more commonly seen however in chronic

Liver changes in monkey malaria

Changes developing in the liver in monkey malaria are remarkably similar to those seen in the human disease and afford an important

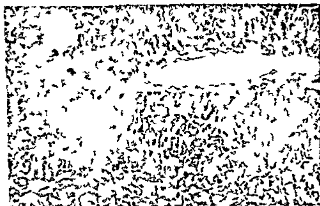


Fig 9—Centrilobular degeneration and necrosis of liver cells in acute *P knowlesi* infection in *M mulatta* Note the dilatation of the central veins and sinusoids and the atrophy and fatty degeneration of the less affected cells Compare with Figs 5 and 7

means of investigating the sequence of events and something of the pathogenesis of the human lesions

Taliaferro and his associates have studied the histopathological changes in the livers of monkeys infected with *P knowlesi* and *P cynomolgi* at various stages of the disease acute and chronic In *M mulatta* killed in the late stages of an acute untreated *P knowlesi* infection they found some cloudy swelling and vacuolation of the liver cells towards the centre of the lobules and numerous transitional mononuclear cells (probably derived from the undifferentiated endothelial cells) and an increase in the activity of the phagocytic cells lining the sinusoids some of which contained pigment In fatal treated or untreated cases of *P knowlesi* infection many of the liver cells were swollen and vacuolated and at the centre of the lobules some were found to be necrotic In the necrotic areas infiltration with polymorphs was observed in some animals The Kupffer cells were tremendously swollen and contained pigment and red cells both parasitized and unparasitized In monkeys which had died from a relapse of *P knowlesi* infection some time after treatment of the primary attack the liver cells in the centre of the lobule showed all stages of degeneration sometimes very

P knowlesi for some time These authors suggest that in human malaria round cell (cells of the lympoid type —Taliaferro and Mulli

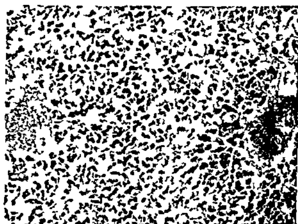


FIG 8—L er from a case of malignant tertian mal ria show g dense accumulations of small lymphocyte cells in the portal r gions Note also the atrophy of the liver cells and d ruption of the cell columns

gan 1937) infiltration is more obvious in patients with long malarial histories This is not always the case however since accumulations of round cells have been commonly reported in acute primary attacks of *P falciparum* malaria (Gaskell and Millar 19_0 Dudgeon and Clarke 1919) Taliaferro and Mulligan believe that round cell infiltration about the portal vessels may represent a reaction to the malarial infection and point out in support of this that such mantling occurs at the time of the crisis in birds infected with *P cathemerium* Klotz (19_9) however considers that such cellular infiltration is entirely non-specific and not essentially related to the malarial infection

The latter author described another form of infiltration which is sometimes seen in malaria Here the cells usually round cells are found in the substance of the lobule especially towards the periphery and in the boundary zone between the necrotic tissue and the relatively healthy cells of the outer zone According to Klotz such infiltration is not found in the central necrotic regions Occasionally especially in association with severely necrosed tissue a proportion of the infiltrating cells may be polymorphs sometimes pigmented Infiltration with polymorphs alone has been described (Dudgeon and Clarke 1917)

the pathogenesis of the liver lesions Rigdon and Stratman-Thomas (1942) reviewed some of the relevant literature in a discussion on the pathological lesions seen in *P knowlesi* infections of *M mulatta* They pointed out that the heart muscle in both human and monkey malaria is often pale flabby degenerate and even necrotic and the heart cavities sometimes dilated especially on the right side They concluded that the liver changes were secondary to congestion following cardiac failure

In cases in which heart failure is evident the above explanation of the pathogenesis of the liver lesions may be correct or partly correct but there are many fatal cases of malaria and blackwater fever in which cardiac failure never occurs or appears only in the very last stages of the illness Such patients frequently die in a condition of vascular collapse very closely allied to shock in which right sided heart failure does not occur and where the venous return to the heart is markedly diminished so that back pressure effects originating in the vena cava cannot have any part in producing the liver lesions Nevertheless at autopsy severe liver changes are often found which are indistinguishable from those described by Gaskell and Millar and many others (Rigdon 1942 Maegraith 1944 Diggs 1945 Crile 1921 Wiggers 1918 Dale 1919 Mann 1919 Cannon 1923 Moon 1938) It is clear therefore that in such cases other explanations of the vascular phenomena seen in the liver are called for In this connection it is important to note the views of Moon who states The similarity to passive congestion accounts for the failure of pathologists to recognize the visceral changes of shock A review of necropsy records in cases of shock shows that the circulatory changes were adequately described but invariably they were interpreted as passive congestion

Dilation and congestion of the small vessels of the liver may result either from some form of obstruction to venous flow or from changes in the vessels themselves Obstruction to the venous flow may be produced as described above by back-pressure from a failing heart but it may also occur in other ways with the development of liver lesions very similar to those of cardiac failure For instance when the venous outflow of the liver is suddenly blocked such lesions may be produced in the course of a few days This is well seen in the syndrome of Chiari s disease a condition in which the orifices of the large hepatic veins are obstructed by thickening of the intima by vascular canalized fibrous tissue or sometimes by thrombosis When such obstruction is acute the liver cells may be completely destroyed except for those near the periphery of the lobules There is intense

severe including fatty degeneration and necrosis In the necrotic areas there were accumulations of polymorphs The changes in Glisson s capsule and the vessels therein have already been described (Taliaferro and Mulligan 1937)

PATHOGENESIS

The most striking and constantly reported features of the hepatic lesions of malaria and blackwater fever are apart from pigmentation the atrophy degeneration and necrosis of the polygonal cells in the central zone and the dilatation and frequent congestion of the sinusoidal vessels and central veins of the lobules

In many ways the lesion closely resembles that seen in right-sided heart failure with its attendant interference with the escape of venous blood from the liver and so-called back-pressure effects (which probably arise from cellular anoxia and not pressure) Lesions associated with cardiac failure usually progress more slowly than those in malaria and do not often reach the extreme stages of necrosis seen in the latter disease Nevertheless in some cases of malaria and blackwater fever the effects of a failing heart probably contribute to the ultimate pathological picture According to Gaskell and Millar (1920) in cases of malignant tertian malaria in which death followed cardiac failure (their cardiac group of patients) liver lesions occurred but were mild in type and characterized by separation of the columns of hepatic cells with atrophy and some degree of degeneration and necrosis chiefly in the cells of the central zones of the lobules The capillaries (? sinusoids) were distended with blood again mainly in the central zone In cases with extensive parasitaemia and in which the symptoms of heart failure were never evident until the end (the septicaemic group) the liver lesions were severe and the congestion of the vessels in the central zone of the lobule was marked the separation of the cell columns in this region being often accentuated by the marked back pressure congestion In fatal cases of cerebral malaria with no obvious signs of heart failure the liver lesions were also well developed in the usual pattern although the congestion was not pronounced

It can be inferred from the account of the lesions observed by Gaskell and Millar that these authors consider that in some cases cardiac failure was chiefly responsible for the liver lesion They do not however attempt to explain the development of the almost identical hepatic changes seen in the absence of heart involvement Other authors have also implicated heart failure and venous congestion in

macrophages must result from interference with the drainage of the venous blood from the lobules Congestion of the liver has been frequently described in human cases of surgical shock (Moon) In acute serum shock in dogs it is one of the most striking phenomena In the latter case within a few minutes of the administration of the trigger dose and coincident with the general fall in blood pressure and decrease in cardiac inflow the liver begins to swell and rapidly becomes turgid with blood On section the vessels are tremendously congested with the maximal effect noticeable in the central region of the lobules It has been shown experimentally that the venous outflow from the liver is reduced in such anaphylactic shock (Simonds and Brandes 19-7)

Since the inflow of blood to the liver is apparently not greatly increased in the shocked dog the congestion of the organ is presumably due to active reduction of the venous outflow arising from obstruction somewhere in the hepatic venous tree Similar obstruction may be brought about by the injection of histamine (Baer and Rossler 1927 Mautner and Pick 19-9 Bauer *et al* 1932) peptone (Simonds and Brandes 19-9) and digitalin (Katz *et al*)

Bauer Dale *et al* (193-) investigated the flow of blood through the perfused dog s liver under a variety of conditions and obtained clear evidence of the existence of a mechanism which controlled the outflow This mechanism was thrown into intense activity by histamine and relaxed by small doses of adrenalin or stimulation of the sympathetic nerves to the liver They showed experimentally that this sphincter mechanism was located near the caval ostia of the larger hepatic veins where the venous walls are especially muscular in the dog (Arey and Simonds 19-0 Bauer *et al* 193-) They were unable to demonstrate similar sphincter activity in cats and goats Small doses of adrenalin however cause an accelerated venous flow from the liver in most species associated with a reduction in organ volume (Franklin 1937) Stimulation of the splanchnic nerves may also have the same effect

A similar sphincter in the hepatic veins of man has not been demonstrated physiologically but Elias and Feller (19-6) found constrictions in the caval ends of the hepatic veins when hot formalin was injected into the vena cava of fresh human cadavers Section of these veins showed that the plain muscle coat increased in thickness near the opening of the vein where it intermeshed with the muscle layers of the vena cava Miyake (19-9) has reported a thick layer of longitudinal muscle fibres in the larger hepatic veins in man which he

congestion of the central vein and its tributary sinusoids and extensive haemorrhage into the parenchyma In cases of more gradual development extensive atrophy of the parenchyma in the centres of the lobules occurs with the living cells confined to the periphery The sinusoids are distended and there may be haemorrhages Regeneration of liver cells and increase of interstitial tissue are also described Thompson and Turnbull describe the pathological findings in this syndrome thus At autopsy the remarkable association of a liver showing extreme atrophy with venous congestion with a heart in which there is no evidence of chronic embarrassment immediately suggest the site of the lesion i e occlusion of the hepatic veins (Chiari 1899 Thompson and Turnbull 1912)

The acute syndrome of Chiari s disease illustrates clearly the effects of active obstruction to the venous outflow of the liver and provides a remarkable instance of the production of a liver lesion fundamentally similar to that seen in heart failure but occurring in a clinical condition in which the heart is primarily unaffected Such a picture helps in a way to explain the pathogenesis of the liver lesions in malaria since although thrombosis or other intensive obstruction of the hepatic veins have not been described in malaria it is clear that the liver lesions seen in that disease might arise from any form of active impedance of the hepatic venous flow The available evidence, as we shall see points to venous obstruction being in fact the most likely aetiological factor at work in the pathogenesis of the liver changes in malaria The appearance of centrally placed lobular degenerative lesions following Ecks fistula and ligature of the portal vein or even (sometimes) the hepatic artery might seem at first to invalidate this statement but it is possible that the changes arising in such experiments are determined by a reflex obstruction to the hepatic venous flow initiated by the operative procedure (Bainbridge and Leathes 1906 Rous and Larimore 1920 Behrend *et al* 1922 Cameron and Mayes 1930)

The possible existence of some form of active obstruction to venous flow in the liver is most easily pictured in those cases in which the clinical condition approaches that of shock e g algid malaria or some cases of blackwater fever Here the liver lesion displays the picture of severe venous congestion developing contemporaneously with a general condition in which there is vascular collapse and a reduction in venous return to the heart Under such circumstances congestive back-pressure effects must be impossible so that any marked degree of congestion as distinct from thrombosis stasis or dilation of small vessels arising from mechanical obstruction such as hypertrophied

The drainage of the liver lobules and the blood flow through them which is normally sluggish except on the venous side may also be impeded by changes in the sinusoidal vessels themselves As has already been shown above the hyperplasia and hypertrophy of the littoral cells accompanied by cytoplasmic swelling and intense phagocytic activity may give rise to mechanical obstruction of the blood flow by reducing the effective bore of the vessels Mechanical obstruction of blood flow may also result from accumulation of free phagocytes or masses of leucocytes or red cells either in a state of sludge agglutination (Knisely 1945) or forming a thrombus These phenomena are uncommon in human malaria but the potentiality of a further form of obstruction that of stasis of erythrocytes exists in any conditions in which the cells of the vessel walls are liable to damage As Moon (1938) points out once the vascular wall is damaged stasis may be expected especially if there is associated vascular dilatation since the permeability of the endothelial membrane is increased and unphysiological quantities of fluid escapes into the tissues so that the red cells tend to become packed firmly in the lumen of the affected vessel Such stasis has not commonly been reported in the liver in malaria although it is often seen in other organs especially the brain Its absence from the liver is possibly associated with the fact that the permeability of healthy liver capillaries and sinusoids is much greater than in most other tissues so that fluid resembling plasma normally passes directly through the vessel walls to bathe the tissue cells (Moon 1938 Drinker and Field 1933) Damage to the endothelial lining of hepatic vessels is therefore unlikely to give rise to great alterations in the passage of fluid from the lumen to the surrounding tissues a fact which may possibly explain the rarity of obvious oedema in even the severest hepatic lesions In any case the lymphatic drainage from the liver is highly efficient and excess fluid passing from the blood vessels to the tissues would be speedily removed as long as the lymphatic flow were not itself affected It is of course possible that fluid is in fact passing in abnormal volumes from the liver sinusoids into the tissues in malaria especially in degenerated areas and that the escape of this fluid may in itself be so great as to impede the flow through the vessels Under such circumstances however the development of stasis would appear inevitable even in severe anaemia when the numbers of corpuscles in the sinusoids were greatly reduced Such stasis as we have said does not occur and it is unlikely that the prevailing anaemia could effect its appearance since although absent in the liver it may be very much in evidence in other organs in the same patient

compares with those of the vena cava and Elias and Feller (1931) 'spur like muscular processes projecting into the lumen of the hepatic veins near their orifices

It is possible therefore that some sphincter mechanism exists in the hepatic veins in man similar to that in the dog capable of obstructing the flow through the venous vessels of the liver This obstruction to flow might arise either directly or indirectly by interfering with the drainage of what Deysach (1941) calls the 'small sluice vessels (which open directly from the sinusoids into the sublobular veins) thus diverting a larger volume of blood than normal through the central lobular veins and congesting them Deysach (1941) has produced experimental evidence of the existence of these sluices in certain animals in which the hepatic veins are not richly supplied with smooth muscle but his work has not yet been confirmed

If such a hepatic venous sphincter mechanism is present in man it presumably reacts to shock and histamine by constriction and may well account for the hepatic lesions seen in severe malaria It is however dangerous to argue from the dog to man and the hypothesis needs experimental proof An analysis of hepatic blood flow by means of intravascular injection of X-ray opaque fluids would probably provide important information

Knisely (1939) has described sphincter mechanisms at the efferent ends of individual sinusoids in the frog s liver capable of regulating the amount of blood passing through the sinusoid and although (apart from Deysach s work) nothing of this kind has so far been identified in mammals intermittent flow through sinusoids has been observed in rabbits rats and mice by Wakim and Mann (1942) These authors found that sinusoids were sometimes packed with motionless red cells and were sometimes clear containing practically none These observations suggest the possibility of there being intrahepatic mechanisms capable of controlling the sinusoidal blood flow If this proves to be the case interference with such mechanisms might bring about gross changes in circulation within the lobule and possibly play a part in the pathogenesis of liver lesions such as those seen in malaria There is no information on this point Indeed a great deal of experimental work is needed before we can begin to understand the normal circulation through the liver quite apart from that occurring in pathological conditions It seems clear however that the congestion of the liver in malaria may be accounted for in some cases by active interference with the venous drainage Such interference if persistent could as we shall see give rise to secondary changes in the parenchymal cells

acute heat collapse has been commonly demonstrated (Caplan 1944 etc) and Kopp and Solomon (1937) have reported clinical states closely resembling shock occurring in human cases in which hyperthermia was induced by exposure to hot moist air Hartman (1938) has described in detail pronounced alterations in cerebral blood flow resulting from fever in humans and dogs and Moon (1938) after reviewing the literature states that exposure to heat above a certain level causes disturbances in circulation originating in the first instance in the capillaries and giving rise to congested conditions of viscera and degeneration both in the brain and the parenchymatous organs

The two processes chiefly involved in the development of pathological changes in the liver parenchymal cells in malaria are probably anoxia with *consequent interference with oxygen supply to the cells* and some kind of direct toxic action on the cells caused by non specific diffusible substances produced directly or indirectly during the course of the disease

The vascular changes which develop in the liver in malaria lead eventually to appreciable slowing of the hepatic circulation and give rise to progressive stagnant anoxic conditions (Peters and van Slyke 1931) with marked disturbances of the gaseous interchanges between the blood and the parenchymatous cells and vice versa When the oxygen supply to the cells is reduced below the optimal tension necessary for full metabolic activity the cells become damaged and if the anoxic conditions persist the damage may become irreversible and go on to degeneration and necrosis The speed with which these changes develop depends partly on the degree of anoxia obtaining locally and partly on the prevailing metabolic level at which the cells are attempting to function The latter may be considerably raised above normal in malaria particularly in hyperthermic cases and when such increase in metabolism occurs the demand for oxygen increases with it and signs of oxygen deficiency develop correspondingly rapidly The effect of such stagnant anoxia *in malaria is likely to be* considerable as will be appreciated from the histological picture presented in severe cases and from the fact that in conditions other than malaria e g continued right heart insufficiency severe liver changes may arise from circulatory disturbances only

Intralobular circulatory retardation is however only one of several factors in malaria leading to the creation of anoxic conditions in the liver parenchymal cells Another factor of considerable importance is the invasion of the red cells by plasmodia In the individual erythrocyte this invasion is accompanied by an appreciable loss in haemoglobin

The intravascular agglutination of erythrocytes so common in severe malaria and the production of a circulating sludge of massed corpuscles described by Knisely and his associates and by Lack in the malaria of man monkeys and birds should provide ample opportunity for the development of stasis and thrombosis in a vascular system markedly obstructed by mechanical means such as hypertrophy of the lining cells of the vessels The fact that in most cases of malaria there is little or no evidence of either stasis or thrombosis indicates that mechanical obstruction can play only a very small part in the impedance of blood flow through the lobules

It therefore appears that neither the mechanical effects of hypertrophied cells in the vessel walls nor the severe damage to the endothelial lining leading to dilatation and increase of permeability can be considered major factors *per se* in the impedance of the circulation through the liver lobule in malaria and blackwater fever

Active obstruction to the hepatic venous flow thus seems to be the most reasonable interpretation of the vascular phenomena (Maegraith and Findlay 1944) The mechanism whereby such obstruction arises is not yet clear apart from an element due to right heart failure and possibly some kind of active constriction of the sublobular interlobular and hepatic veins as suggested above

We are also completely in the dark as to the initiating factor in these conditions It may be directly related to the malarial infection For example there may be some soluble chemical substance—the so-called toxin as yet unidentified—elaborated by the parasite which diffuses into the tissues and acts on both vascular endothelium and hepatic cells Metabolites produced by the parasite may act similarly and the products of schizogony liberated suddenly into the general circulation may be involved Malarial pigment has also been incriminated On the other hand since liver lesions occur in malaria or blackwater fever in the presence of very scanty parasitic invasion and similar lesions appear in conditions in no way allied to malaria especially when associated with clinical shock it is more likely that some general factor is ultimately involved such as tissue anoxia which leads primarily to circulatory changes in the liver lobules in much the same way as they may develop in the kidney in the syndrome of renal anoxia It is interesting to recall here the frequent association of hepatic and renal lesions of this type a fact which suggests a possible common fundamental mechanism In cases exhibiting high fever some circulatory changes may also be brought about by the high blood temperature Thus the similarity to surgical shock of clinical conditions such as

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content and a consequent reduction in the potential concentration of oxyhaemoglobin available for carriage of oxygen (Christophers and Fulton 1938) The growth and development of the parasite in the substance of the erythrocyte further draws on the oxygen of the oxyhaemoglobin with the result that still less becomes available for ultimate distribution to the tissues The parasitized red cell must therefore be considered as a poor oxygen carrier and thus in patients with high parasitaemia there will be a correspondingly gross limitation of the oxygenation of the tissues It is not unlikely that the oxygen carrying powers of the non-parasitized erythrocytes may also be reduced in malaria not because of loss of haemoglobin but as a result of inadequate oxygen saturation or interference with the dissociation of the oxyhaemoglobin Fever may play a part in such processes since Barcroft has shown that the total oxygen saturation of the blood is decreased as the blood temperature rises An increase in cell metabolism also accompanies rising temperature the basal metabolic rate accelerating by as much as 40 per cent above normal when the temperature reaches 106° F so that concomitant with the reduction of oxygen saturation and consequent decrease in oxygen availability to the tissues there is an increased demand for oxygen tending to worsen the existing anoxic state (Hartman 1937 Barcroft 1920) The oxygen carriage by erythrocytes and the dissociation of oxyhaemoglobin have not been very thoroughly studied in malaria although these matters are clearly of great importance to the understanding of the pathological processes of the disease It is possible that both may be interfered with by diffusable substances produced directly or indirectly by the parasite but so far no evidence has been brought forward to this effect It may be that the persistent failure of all workers to discover anything in the nature of a parasitic toxin in malaria or blackwater fever is due to the fact that such substances have not been sought in the right place Further work on the carriage of oxygen and the dissociation of oxyhaemoglobin both in parasitized and unparasitized cells in malarial infections might be well repaid

The existence of severe anaemia will also give rise to anoxic conditions in the liver cells by limiting the available oxygen in the circulating blood and by the abnormally great fall in oxygen tension of the blood following removal of the usual volume percentage (Peters and van Slyke 1931 Hartman 1937) Rich (1930) states that atrophy of the hepatic cells about the central vein of the lobule commonly occurs in pernicious anaemia and in certain secondary anaemias Simple haemorrhage in dogs may have the same result The lesions

found in anaemia even in the absence of heart failure often closely resemble those arising in so-called passive venous congestion of the liver associated with failing heart He suggests that in each case the tissue changes are primarily due to anoxia It is difficult to produce such anaemic anoxia experimentally by bleeding except by removal of relatively large volumes of blood or gross reduction in the number of circulating cells so that it is only in the severest malarial anaemias that this form of anoxia is likely to be of much significance

It is possible by various chemical means to damage liver parenchymal cells e g by the use of cyanide or chloroform Under such circumstances the damage may be very severe and arise from direct chemical interference with metabolic processes In many cases the fundamental derangement is one in which the cell loses its power to utilize the available oxygen a condition which Peters and van Slyke (1931) have called histotoxic anoxia Various substances have been found to have this property including certain narcotics—alcohol barbiturates—and Keilin has shown that the underlying process is one whereby the oxycytochrome of the tissues becomes stabilized so that oxygen cannot readily be removed from it Such processes may also be working in malaria since there is great similarity especially in the brain between the lesions produced by histoxic anoxia and those of malaria (Hartman 1937 1938 Jowett and Quastel 1937 Rigdon 1942) They may result from the presence of specific toxic substances not yet identified or from the accumulation of metabolites such as for example pyruvic acid derived from cellular or parasitic activity the latter especially under conditions in which the circulation and thus the drainage of the area concerned are impeded

It has been said above that the lesions in the liver in malaria may arise either from anoxia or poisoning alone or from a combination of the two processes It is most likely that anoxia is as a rule the more important process for it is not only capable of producing very similar lesions—for instance under conditions of venous congestion—but it also gives rise to severe damage to the vascular walls which once started may progress steadily especially as the vessels are themselves to some extent affected by the condition of the parenchyma (Moon 1938) At this stage in our knowledge however it is not possible to rule out direct toxic effects on the cells and the search for possible toxic agents must be continued

The degenerative and necrotic changes in the parenchyma account for the deviations in function seen in the severest cases but as has been said elsewhere no such anatomical changes may be visible even in

cases in which gross variations in so-called hepatic function tests have been found Some of these latter deviations in function probably arise from reversible changes in the liver cells resulting from mild degrees of anoxia It has been shown for instance that anoxic conditions bring about alterations in the permeability of the hepatic cell membrane to inorganic ions

The rise in blood potassium which occurs in asphyxia or haemorrhage was found to be prevented by hepatectomy and adrenalectomy in the former case and by hepatectomy only in the latter Houssay and his collaborators argued from this that the anoxia in asphyxia stimulated the adrenal to produce adrenalin which in turn mobilized the potassium from the liver in haemorrhage the effect appeared to be directly hepatic (Mullin Dennis and Calvin 1938 Houssay Marenzi and Gerschman 1936 1937) Mobilization of liver glycogen also occurs in anoxic states and there is evidence that the increase in blood sugar depends on the degree of anoxia present (van Liere 1942) It has been shown recently that during the hepatic anoxia resulting from anoxic anoxia and shock produced by crush injuries almost complete failure of liver glycogenesis occurs At the same time normal glycogenolysis continues The findings of Sinton and Hughes and of Williams in regard to the failure of the liver to deal with ingested laevulose are particularly interesting in this connection

Rich believes the development of jaundice in cases of pulmonary infarction arises from the depression of bilirubin excretion consequent on a state of anoxaemia Such depression has been demonstrated in artificially induced anoxaemia in human subjects and animals following the injection of bilirubin or laked blood (Rich 1930 Harrop and Barron 1931) A decrease in the excretion of bilirubin has also been reported in severe anoxic anoxia in rabbits It will be noted that these changes in hepatic function caused by anoxia are in each case paralleled in malaria where there may be an increase in plasma potassium corresponding to the paroxysm and similar changes in blood sugar and where there is frequent evidence of bilirubin retention

Anoxia also greatly affects the vascular endothelium increasing its permeability to protein and hence increasing the escape of fluid The possibility of such changes occurring in the vessels of the liver has already been discussed and it has been pointed out that increasing the permeability of the hepatic vascular endothelium may not affect the surrounding tissue as much as in other organs because the natural permeability of the hepatic vessels is considerable and the lymphatic drainage of the liver is normally an efficient one The latter fact may

also explain why in even severe anoxic states the water content of the liver is not usually increased (van Liere 1942)

Anaphylactoid shock in some way related to the periodic nature of the parasite's asexual cycle may also be concerned in the pathogenesis of the hepatic lesions in malaria According to some workers the foreign protein and debris thrown into the blood stream at schizogony may slowly sensitize the patient's tissues until a point is reached at which antigen-antibody reactions become sufficiently pronounced to damage the cells A further possibility is that the chemical structure of the liver cells becomes altered by malaria or anoxia in such a way that the tissue becomes antigenic to itself and consequently suffers injury It is well known that the injection of homologous tissue antibodies into animals will produce violent tissue reactions in the recipient Thus injection of rabbit kidney-antiserum (prepared by injection of rabbit kidney tissue into another species of animal) will give rise to acute fatal nephrotoxic changes closely resembling acute nephritis Schwentker and Comploier (1939) have shown that such homologous antibodies can be produced in rabbits by injection of a mixture of homologous tissue plus extracts of certain bacteria especially staphylococci This means that in staphylococcal infections in rabbits antibodies to rabbit kidney tissue may be produced in the animal's body the antigen being derived from kidney cells damaged by the bacterial infection Gear (1946) has suggested that a similar reaction may account for haemolysis in blackwater fever It is conceivable that some such process goes on in the liver and that as a result of the malarial infection or of the anoxia arising from it the liver cells may become autoantigenic and give rise to noxious liver autoantibodies Support for such a hypothesis is given by the further observation of Gear to the effect that liver tissue from monkeys dying of yellow fever is auto antigenic and gives rise to anti liver cell bodies when injected into other monkeys of the same species It is difficult to estimate the part played by such processes in malaria and at the moment it is not easy to fit them into the pathogenesis of the localized hepatic lesions that develop in that disease although it is conceivable that they become enhanced in already anoxic tissue

Although as we have seen anoxia in its various forms appears to be the main process involved in the development of hepatic tissue damage *in malaria its effects together with those of the hypothetical* toxic agents mentioned above may be considerably modified by the general condition of the patient particularly his nutritional status Apart from the presence of toxic substances in the food such as the

cyanogenous glucosides of certain root crops general dietary deficiencies may in some cases initiate and sustain liver damage For instance Gilbert and Gillman (1944) found that extensive liver damage occurred in albino rats fed for long periods on the typical corn diet of the South African negro Experiments of this type give little more than general information concerning the part malnutrition and deficiencies of one kind or another may play in the genesis of liver damage especially in poorly fed indigenous populations and it is not possible here to go into this question in any detail It is important however in connection with liver injury in malaria to consider the state of the diet so far as its content of protein is concerned Both carbohydrate and protein have been shown to have some protective action in regard to the toxic effects on the liver of certain chemical substances e g arsenicals and chloroform and it has been found in the case of the latter that as the protein stores of the animal are *diminished* so the susceptibility to chloroform is increased Glyn and Himsworth (1944) have produced massive hepatic necrosis in rats by severe dietary protein deficiency and have shown that such injury can be prevented by the restoration of protein to the diet or by the administration of the sulphur-containing amino acid methionine The latter has also been found effective in protecting the liver against the toxic activity of arsenicals (Goodell *et al* 1944) and certain infections such as leptospirosis in guinea pigs (Wylie 1946 Miller and Whipple 1940 Davis and Whipple 1919 Gyorgy and Goldblatt 1939)

It is thus clear that protein deficiency especially if associated with specific deficiency of essential aminoacids such as methionine may expose the patient to the risk of serious liver damage Such protein deficiency occurs commonly in malaria It has been mentioned elsewhere (Chapter IV) that in this disease as in blackwater fever there is a decided change in the blood protein content especially so far as the albumin concentration is concerned Albumin is synthesized in the liver so that the concentration of this protein will fall as the functions of the liver are depressed The rapidly developing and increasing hypoproteinaemia of malaria may thus help to initiate a vicious circle which ultimately results in very severe hepatic dysfunction and injury Protein may also be deficient in the individual malarial patient as a result of reduction in intake during the acute disease or lack of food protein following anorexia (itself liable to exacerbation by protein or methionine deficiency Himsworth 1946) vomiting or diarrhoea The raised metabolism induced by fever may further increase the relative deficiency of protein and so aggravate the con-

dition Finally in badly nourished individuals the protein store may be low and rapidly depleted by the disease so that the effects of protein deficiency will develop early and severely and when undernutrition reaches the stage of starvation the absorption of amino acids from the gut may itself become affected and lead to acute shortage of methionine and other essential amino acids including lysine How great a part is played in any individual case of malaria by such nutritional factors may be very difficult to assess but in badly fed indigenous populations in endemic malarial regions these factors probably become increasingly important and must not be overlooked

Glenn *et al* (1946) have studied this question of the influence of protein and other deficiencies in induced malaria They investigated liver function tests in three groups of 20 neurosyphilitic patients 15 of whom were given *P vivax* and five *P malariae* infections The first group received routine treatment the second group were given extra protein plus vitamins A B complex C and D together with liver injections the third group were treated like the second but had in addition thiamin and intravenous glucose therapy Clinically the malarial attacks appeared more severe in groups two and three than in the first group The results of organ function tests were much the same in all groups The authors concluded that the deviations of liver functions were not influenced by the extra protein and vitamins

The state of the liver itself at the time of the malarial attack is also an important factor in the pathogenesis of hepatic lesions Various concomitant diseases may for instance be dormant in the tissues and cause complications by becoming active as the malaria develops This must always be remembered in assessing liver dysfunction and injury in populations living in regions in which yellow fever or infective hepatitis are endemic It is also of great importance in therapeutic malaria in syphilitic patients for in such cases interpretation of results must be made with the reservation that previous or present hepatic damage by syphilis or therapeutic arsenic may affect the picture O Leary *et al* point out in this connection that the jaundice seen in syphilitics after administration of malaria may be the result of the direct action of the malaria on the liver which they consider most likely or of the activation of syphilitic hepatitis which has been lying dormant or even some entirely secondary infective agent In some cases there is evidence that liver injury develops more readily in patients previously affected by arsenic For instance Wile and Sams (1934) have described jaundice developing during malarial therapy in a patient who had previously had post arsphenamine jaundice Kopp

and Solomon (1943) give an account of a patient in whom hepatic injury had arisen from prolonged arsenic treatment and in whom generalized jaundice developed during malarial therapy

Many similar cases have been described Nevertheless where liver function tests have been followed in therapeutic malaria it has been found that they return to normal values rapidly after the treatment of the malaria both in patients who have had previous arsenical treat ment and in those who have not and Fredricks and Hoffbauer have reported deviations in hepatic function tests in a case given therapeutic malaria more than 10 years after arsenic treatment in which liver biopsies showed no gross anatomical changes Moreover many workers have reported negative findings in control cases of syphilis in regard to liver function tests and Williams has stated that no changes in laevulose tolerance tests occurred in syphilitics treated with relapsing fever It therefore seems reasonable to consider such deviations of hepatic function to result primarily from the malaria but it is possible that previous damage to the liver cells may make them more susceptible to any form of injurious agent This has been shown to be the case in syphilitics treated with malaria and subsequently with arsenicals Wile and Sams (1934) described two cases in which jaundice developed during malarial therapy and again during arsenical treatment Other drugs besides arsenicals may predispose the liver cells to damage in malaria but this does not seem to be the case with the usual antimalarial drugs

RECAPITULATION

Liver lesions in malaria and blackwater fever as examples of an hepatic syndrome of wide distribution

Signs of liver involvement may appear at any stage in malaria or blackwater fever They are commonest in *P falciparum* infections and present in various degrees a syndrome of enlargement of the liver and jaundice with or without bilirubinuria Jaundice may be accompanied by the indirect biphasic or direct van der Bergh reaction Deviations in serial hepatic function tests occur even in the absence of overt clinical signs of hepatic dysfunction

Biopsies of the liver during acute attacks of malaria associated with deviations in function tests may show no structural changes but in fatal cases pronounced pathological lesions are common The histological picture is essentially one of congestion and central zonal degeneration necrosis and atrophy of the liver cells The central

veins of the lobules and tributary sinuses are congested or in anaemic cases dilated and empty Stasis and thrombosis and other forms of mechanical obstruction to the capillaries and sinuses are uncommon There is intense phagocytosis of parasitized and unparasitized erythrocytes and haemozoin by the reticuloendothelial cells which are often hypertrophied

The lesions resemble those found in right heart failure which arise as a result of anoxia brought about by interference with the escape of venous blood from the liver Heart failure is not common in malaria and hepatic lesions often appear in cases in which the clinical condition is one of shock with a restriction of venous flow to the heart In these cases there can be no question of passive venous congestion Since obvious interference with intralobular blood flow resulting from changes in the vessels themselves is not a prominent feature of the pathological picture some other form of impedance to the circulation must be postulated The most probable explanation is active obstruction to the hepatic venous outflow produced by changes in the venous blood vessels Reduction of this outflow would create a condition of stagnant anoxia in the liver similar to that seen in heart failure The effects of obstruction to the venous blood flow of the liver are demonstrated in Chiari s disease in which there is extensive mechanical resistance to venous outflow but no heart failure The changes in the hepatic tissue in malaria probably result primarily from stagnant anoxia developing because of interference with intralobular blood flow Contributory factors include possible malarial toxins and by-products of plasmodial metabolism and autoantigenesis of the liver cells Specific activity of the parasite or its products is unlikely since most severe lesions may develop in the absence of plasmodia e g in blackwater fever The central distribution of the lesion may result from the removal of oxygen from the already anoxic blood by the peripherally placed cells specific sensitivity of the central cells to anoxia or unidentified diffusable chemical substances or some rearrangement of the intralobular blood flow

Lesions similar to those in malaria and blackwater fever are found in many other conditions such as chloroform poisoning (Whipple and Sperry 1909) heart hyperpyrexia (Malamud *et al* 1946) severe burns (Hartman *et al* 1938 1943 Wilson *et al* 1938 Erb *et al* 1943) post-operative necrosis of the liver (Sutton 1943) shock (Moon 1938) pernicious and secondary anaemias (Rich 1930) effects of exposure to anoxic anoxia (Rosin 1928 Rich 1930) and experimental interference to the blood supply to the liver in animals (Whipple

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CHAPTER VII

THE KIDNEY IN MALARIA AND BLACKWATER FEVER

(1) *Clinical signs of dysfunction Pathology*

PROTEINURIA RENAL DYSFUNCTION IN ACUTE MALARIA AND BLACKWATER FEVER RENAL DYSFUNCTION IN CHRONIC AND RECURRENT MALARIA PATHOLOGICAL CHANGES ( ) Changes a[illegible] g d[illegible] ctly f[illegible]om mala[illegible]ial inf[illegible]ct[illegible]n P[illegible] sites — P[illegible]g[illegible]ent[illegible]tion and ph[illegible]gocyto[illegible] — Vascul[illegible] [illegible]hanges (ii) Changes [illegible]n the paren[illegible]hyma ( ) A[illegible]ute mal[illegible] (b) Ch[illegible]on[illegible] and r[illegible]ent m[illegible]al[illegible]ria ( ) Chang[illegible] [illegible] bl[illegible]ckw[illegible]t[illegible]r f[illegible]ver M[illegible]c[illegible]o[illegible]op[illegible]c [illegible]pp[illegible]n[illegible]es — H[illegible]tolog[illegible] [illegible] [illegible]hanges ( ) Gl[illegible]meruli (b) Tubules ( ) Oth[illegible] tis[illegible]ues — Gener[illegible] [illegible]p[illegible]tu[illegible] in bla[illegible]kw[illegible]ter f[illegible]e[illegible]

CLINICAL evidence of renal dysfunction has been reported in all common forms of human malaria at all stages of the disease Indications of dysfunction during an attack of malaria can be conveniently divided into three groups of syndromes (i) the passage of protein in the urine (ii) the appearance in the acute stages of the attack of a syndrome resembling acute nephrosis usually associated with signs of water retention and occasionally with azotaemia and (iii) the development in chronic recurrent or repeated malaria infections of a syndrome resembling subacute or chronic nephrosis of an hydraemic type

PROTEINURIA

The passage of protein in the urine usually referred to as albuminuria is a frequent occurrence in malaria Proteinuria in the absence of accompanying casts or red cells may not at first sight appear to be very substantial evidence of derangement of renal function In fact Meleney (1941) states that kidney function is not usually disturbed in clinical cases of malaria and apparently regards albuminuria as unimportant when not associated with derangements of water salt or nitrogen secretory functions Proteinuria is often present more over in fevers other than those associated with malaria and in healthy individuals occurs under physiological conditions as MacLean (1919) and Diehl and McKinlay (1935) have shown In order to correlate proteinuria with the malarial attack it must therefore be demonstrated to occur in such attacks considerably more frequently than it does in normal subjects (about 4–6 per cent of subjects)

and Hooper 1916 Rous and Larimore 1920 Bainbridge and Leathes *1906 Behrend et al 1922)* Some of these conditions are clearly anoxic states but in others such as chloroform poisoning or severe burning the mode of development of anoxia is not at first apparent Nevertheless the appearance of similar pathological changes in such diverse conditions indicates the existence of a common pathogenic factor The most rational hypothesis is that there is in all these conditions a change in hepatic blood flow leading to reduction of venous escape and the development of stagnant anoxia in the tissues

In the absence of cardiac failure the retardation of blood flow most probably develops from active obstruction to the venous escape arising from constriction of some part of the hepatic venous tree There is evidence in animals of the existence of a constrictor apparatus in the hepatic veins which can be set in operation by either humoral or reflex mechanisms It is suggested that something similar may exist in man

Although Sinton and Lal found the incidence of proteinuria equal in benign and malignant tertian malaria, most other authors have reported a greater incidence in the latter. Craig's results have already been mentioned. Goldie (1930) also found albuminuria commonest in malignant tertian malaria, and So (1941) observed an incidence of 65 per cent in acute malignant tertian cases as compared to 40 per cent in benign tertian. In So's cases showing albuminuria there was no evidence of derangement of renal function as measured by the excretion of chloride and the phenolsulphonephthalein test.

James (1922) states that temporary albuminuria is frequent in induced benign tertian malaria towards the end of a febrile paroxysm. So (1941) confirmed this observation. Boyd and Proske (1941) observed traces of albumin during the course of four out of five cases of induced *P. vivax* infection and three cases of *P. falciparum* infection, one of which developed oedema. Albuminuria was present in two cases of *P. malariae* infection, both of which developed oedema. They found there was a significant association in the *P. malariae* cases between the appearance of proteinuria and depression of plasma albumin and the development of oedema. The proteinuria disappeared on the termination of the infection.

Giglioli (1930) investigated the incidence of renal symptoms during malaria attacks in a mixed population of Negroes, Indians and others treated in hospital in British Guiana, and found albuminuria in about 25 per cent. In over 900 West Indian Negroes not suffering from clinical attacks of malaria, albuminuria was present in 4.5 per cent. Exertion increased the incidence of albuminuria in a group of apparently malaria free labourers from 7–14 per cent to over 24 per cent. Albuminuria was uncommon in new or recent infections in his cases. During a malaria epidemic in 1926 albuminuria was rare in the first three months, when the epidemic was in its acute phase, and became common as the epidemic curve began to decline and the chronic infections assumed the upper hand. The highest incidence in relation to the species of infecting parasite was found in *P. malariae* infections (11 out of 24 cases). In early cases albumin appeared in the urine intermittently during the febrile reaction and was absent in the apyrexial interval. After repeated attacks the albuminuria became constant and other signs of renal involvement developed.

The evidence outlined above demonstrates that proteinuria occurs frequently in malaria. It is usually assumed that the bulk of the protein which escapes from the plasma in this way is albumin, which has a relatively small molecule, but in fact there is little information about

Most authors have recorded some examples of proteinuria amongst their cases of malaria but the incidence reported varies enormously according to the country in which the cases were investigated and the type and severity of the attack Thayer (1909) in America found proteinuria in about 40 per cent of 350 cases of benign tertian malaria Craig (1909) states that proteinuria is so common that it should be considered more as a symptom of the disease than as a complication. In his experience in the Philippines it appeared in about 50 per cent of cases of benign tertian and 65 per cent of malignant tertian malaria Deaderick (1911) also found albuminuria extremely common in *P falciparum* infections On the other hand Hughes and Bomford (1944) found proteinuria in less than 1 per cent of over 850 cases of malignant tertian malaria amongst British troops in West Africa in contradiction to Holmes a Court (1918) who found it in 49 per cent of his West African cases

The variation in the reports of workers concerning the incidence of proteinuria in malaria may be seen in the following examples Rem Picci (1898) in Rome 6 per cent Ziemann (1924) in Italy 18 per cent (malignant tertian) Anders 0 per cent (120 cases parasite species unstated) Cook 75 per cent (100 cases parasite species unstated) Henson (1913) 25–40 per cent (benign tertian cases persisting for a few days)

Sinton and Lal (1924) attempted to assay the sources of error in the recorded incidence of proteinuria in malaria They investigated 57 cases of benign tertian and 410 cases of malignant tertian malaria in Indians who were living on a standard prison diet and in whom the disease was diagnosed and treated early Proteinuria was found in 12 per cent of the benign tertian cases and 14 per cent of the malignant tertian After a discussion of the literature they concluded that the inconsistency of the results of other authors depended to some extent on the number of urine examinations made in individual cases the time during the attack at which the examinations were made the use of drug therapy and finally the possibility of using a selected population They found the appearance of proteinuria irregular and intermittent in any one subject The administration of quinine had no appreciable effect on the incidence of proteinuria but they detected a slight diminution in the severity of the albuminuria in cases treated with alkali in addition to quinine They could establish no relation between the occurrence of proteinuria and the prevailing degree of parasitaemia the clinical severity of the disease the height of fever or splenic enlargement

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Giglioli (1930) investigated the incidence of renal symptoms during malaria attacks in a mixed population of Negroes Indians and others treated in hospital in British Guiana and found albuminuria in about _5 per cent In over 900 West Indian Negroes not suffering from clinical attacks of malaria albuminuria was present in 4 5 per cent Exertion increased the incidence of albuminuria in a group of apparently malaria free labourers from 7–14 per cent to over _4 per cent Albuminuria was uncommon in new or recent infections in his cases During a malaria epidemic in 19_6 albuminuria was rare in the first three months when the epidemic was in its acute phase and became common as the epidemic curve began to decline and the chronic infections assumed the upper hand The highest incidence in relation to the species of infecting parasite was found in *P malariae* infections (11 out of _4 cases) In early cases albumin appeared in the urine intermittently during the febrile reaction and was absent in the apyrexial interval After repeated attacks the albuminuria became constant and other signs of renal involvement developed

The evidence outlined above demonstrates that proteinuria occurs frequently in malaria It is usually assumed that the bulk of the protein which escapes from the plasma in this way is albumin which has a relatively small molecule but in fact there is little information about

its real nature An investigation of the urinary albumin/globulin ratio in malaria might provide valuable information It is probable that the ratio in malaria is low as it is in physiological proteinuria indicating some minor functional change in the glomerular membrane which temporarily permits the passage of large molecules to which it is normally almost completely impermeable As will be seen later there is evidence that this change in permeability arises mainly from intrarenal circulatory changes and is associated to a lesser degree with fever and the progressive anoxaemia of the disease There may also be a specific factor involved

The temporary proteinuria which appears during the acute malarial attack is probably caused as explained above by changes in the permeability of the glomerular membrane There is no evidence at this stage of any involvement of the nephron In acute malaria however the nephron sometimes becomes affected and evidence of tubular damage appears The protein in the urine is now accompanied by casts of the renal tubules and often by red blood cells The concentration of the urine is sometimes reduced and the condition may progress into a state of acute renal dysfunction not unlike nephritis in which there is oliguria or anuria and nitrogen retention This condition sometimes appears as a terminal event in severe *P falciparum* infections Azotaemia in other forms of acute malaria is infrequent (Benhamou Jahier Berthelemy 1921 Ascoli 1915) but it has been reported in acute quartan malaria Marchiafava and Bignami (1903) state that a condition more closely resembling acute nephrosis is commoner especially in benign tertian and quartan malaria in which there is oedema and clear evidence of water and salt retention This syndrome responds rapidly and completely to antimalarial treatment

More commonly the kidney damage shows itself gradually developing in the course of chronic recurrent or repeated infections especially in quartan malaria In this case the derangement is again mainly tubular and is associated with water and salt retention Nitrogen retention is rare except during acute exacerbations of the malaria in which the renal symptoms may occasionally become exaggerated and go on to uraemia In untreated cases the condition is progressive but in the early stages it will respond to adequate antimalarial treatment although less promptly than the acute nephrosis mentioned above Death may occur as a result of the development of uraemia during a recrudescence of the malaria infection but it more commonly results from a state of general anasarca

The clinical manifestations of kidney changes in malaria thus vary

from simple proteinuria to acute or chronic renal failure The pathogenesis of the two forms of renal failure is probably essentially similar but the clinical pictures are sufficiently dissimilar to warrant separate treatment here

RENAL DYSFUNCTION IN ACUTE MALARIA AND BLACKWATER FEVER

Macfie and Ingram (1917) reported that in West Africa acute nephritis in the active stages of malaria appears to be relatively seldom recorded Rogers stated that the urine in uncomplicated cases seldom showed either albumin or other changes associated with renal damage James (1922) on the other hand found that in artificially induced malaria temporary albuminuria was frequent especially towards the end of the febrile paroxysm and noted epithelial and granular casts in the urine in severe malignant tertian infections Most workers have like James observed some evidence of renal disturbance in acute malaria Rem Picci (1898) in Rome for instance described acute nephritis developing during or after an attack of malaria This nephritis was severe or mild in type and occurred in young rather than old people It appeared in about 1 per cent of cases and was commonest in malignant tertian malaria Thayer in America stated that 21 out of 112 cases of acute nephritis treated in the Johns Hopkins Hospital were caused by malaria infections Over 17 per cent of his 758 cases of malaria of all kinds showed albumin and tubular casts in the urine These urinary findings were commonest in malignant tertian malaria.

Craig (1909) reported some form of nephritis in 3 per cent of all his cases of malignant tertian malaria (presumably in the Philippines) Signs of kidney damage were much less common in benign tertian or quartan malaria He stated that evidence of nephritis is to be found in all fatal cases of malaria Casts of renal tubules were found in ‿5 per cent of his malignant tertian cases and 5 per cent of all cases of benign tertian and quartan malaria Despite this high incidence of casts only a small percentage of cases developed clinical nephritis

According to Flensburg (191‿) nephritis was one of the most frequent complications of the epidemics of tertian malaria in Sweden in the last century Deeks (1916) also stated that nephritis was the commonest complication of malaria occurring in the Canal Zone Renal symptoms usually disappeared after canvalescence but occasionally they persisted and eventually caused the patient s death.

Paterni (19‿9) reported mild acute parenchymatous nephritis in

its real nature An investigation of the urinary albumin/globulin ratio in malaria might provide valuable information It is probable that the ratio in malaria is low as it is in physiological proteinuria indicating some minor functional change in the glomerular membrane which temporarily permits the passage of large molecules to which it is normally almost completely impermeable As will be seen later there is evidence that this change in permeability arises mainly from intrarenal circulatory changes and is associated to a lesser degree with fever and the progressive anoxaemia of the disease There may also be a specific factor involved

The temporary proteinuria which appears during the acute malarial attack is probably caused as explained above by changes in the permeability of the glomerular membrane There is no evidence at this stage of any involvement of the nephron In acute malaria however the nephron sometimes becomes affected and evidence of tubular damage appears The protein in the urine is now accompanied by casts of the renal tubules and often by red blood cells The concentration of the urine is sometimes reduced and the condition may progress into a state of acute renal dysfunction not unlike nephritis in which there is oliguria or anuria and nitrogen retention This condition sometimes appears as a terminal event in severe *P falciparum* infections Azotaemia in other forms of acute malaria is infrequent (Benhamou Jahier Berthelemy 1921 Ascoli 1915) but it has been reported in acute quartan malaria Marchiafava and Bignami (1903) state that a condition more closely resembling acute nephrosis is commoner especially in benign tertian and quartan malaria in which there is oedema and clear evidence of water and salt retention This syndrome responds rapidly and completely to antimalarial treatment

More commonly the kidney damage shows itself gradually developing in the course of chronic recurrent or repeated infections especially in quartan malaria In this case the derangement is again mainly tubular and is associated with water and salt retention Nitrogen retention is rare except during acute exacerbations of the malaria in which the renal symptoms may occasionally become exaggerated and go on to uraemia In untreated cases the condition is progressive but in the early stages it will respond to adequate antimalarial treatment *although less promptly than the acute nephrosis mentioned above* Death may occur as a result of the development of uraemia during a recrudescence of the malaria infection but it more commonly results from a state of general anasarca

The clinical manifestations of kidney changes in malaria thus vary

Surbek considered that the acute nephritis of malaria developed from the state of renal reaction. It was characterized by marked albuminuria slight haematuria and oliguria. The urine contained very numerous epithelial granular and hyaline casts as well as erythrocytes. Nausea and vomiting occurred in some cases associated with a slight increase in blood pressure. Of 17 cases of this type 1[illegible] occurred in *P falciparum* infections two in *P vivax* and one in *P malariae* infections. The condition responded well to quinine therapy. Unfortunately he gives no information concerning the previous malarial history of the cases referred to. It is possible that in the quartan and benign tertian cases there may have been some underlying more chronic type of malarial kidney derangement so that the so-called acute nephritis was in reality an acute exacerbation of an existing lesion. This is unlikely in the *P falciparum* infections which may be regarded as genuine examples of acute renal dysfunction arising in malaria.

Recently So (1941) has described three cases of acute nephritis occurring during acute malignant tertian infections in Japanese. Boyd and Proske (1941) found albumin associated with casts in one of three cases of induced *P falciparum* infection and in one of two cases of *P malariae* infection. Oedema occurred in the *P malariae* infection referred to and in one other *P malariae* and one *P falciparum* infection. The *P falciparum* infection in which casts were present did not develop oedema. The authors concluded that some degree of nephrosis developed in these cases. James (1910) and Marchiafava and Bignami (1903) have also described acute nephritis associated with oedema during *P malariae* infections. In the cases described by Boyd and Proske however the total protein and albumin in the plasma were considerably lowered so that the oedema may not have been entirely renal in origin.

Kean and Smith (1944) reviewed the findings in 100 autopsies on cases of *P falciparum* malaria which died between 19[illegible]5 and 194[illegible] in the Canal Zone. They found there had been clinical evidence of anuria in six cases and four had died in uraemia. Their figures show a higher incidence of renal damage than the comparable figures of Seyfarth (1926) who stated that renal failure accounted for less than 1 per cent of deaths from malarial infections.

As will be seen there is considerable difference of opinion amongst workers on many features of acute renal involvement in malaria but certain points emerge from the mass of apparently contrasting evidence upon which there is general agreement. Most authors for instance have found that acute as distinct from chronic renal symptoms are

less than 1 per cent of about 1 000 cases admitted to his hospital in Rome between 1924 and 1928 Albuminuria was present in 10 per cent of benign tertian and 18 per cent of malignant tertian infections Renal symptoms in addition to albuminuria occurred only in *P falciparum* infections including six of 16 cases of pernicious malaria

Giglioli (1930) found no evidence of acute nephritis in his cases in British Guiana although the incidence of albuminuria and chronic nephritis was high In these cases the majority of renal signs appeared in *P malariae* infections

Jansco and d Engel (1931) reviewed the experiences of over 50 years work in a clinic in Hungary and recorded 28 cases of nephritis Fourteen of these were acute and appeared during active *P falciparum* infections The clinical picture was that of typical acute glomerulonephritis with oliguria and urine of high specific gravity containing large amounts of albumin and sometimes blood visible to the naked eye Microscopically the urine contained blood cells and numerous epithelial granular and hyaline casts Some degree of general oedema was invariably present and in some cases there was an elevation of blood pressure The symptoms corresponded very closely to those of the acute nephritis of scarletina Malaria parasites were present in the peripheral blood in all cases and the response of the nephritic symptoms to antimalarial treatment (quinine) was dramatic In some cases diuresis commenced during the first day of therapy in all it appeared by the first afebrile day With the onset of diuresis the oedema disappeared the blood pressure fell and the urine became normal The subsidence of the nephritic symptoms after the administration of quinine was so striking that the authors suggested it was of pathognomic significance In two cases the urinary sediment contained in addition to cells and casts granules of pigment which the authors believed to be haemozoin The erythrocytes present were never parasitized

Among Malay Javanese and Chinese populations of Sumatra Surbek (1931 a b) found that renal reactions and complications during acute attacks of malaria seem to occur with notable frequency He described four clinical types of renal reactions which correspond closely to those already discussed namely (i) febrile albuminuria (ii) renal reaction (marked albuminuria with urine containing hyaline and pigmented casts) (iii) acute nephritis and (iv) *quartana-nephrosis infantum ac adolescentium* which he describes as a subacute parenchymatous hydropic nephritis occurring especially in untreated quartan malaria

(Stephens 1937) is often described as developing suddenly in the course of the disease It is usually recorded as appearing in the form of severe oliguria or anuria In fact however the renal damage has most probably been progressing for some time before any marked reduction in urinary output occurs

The passage of haemoglobin in the urine is always associated with the presence of protein and granular and hyaline tubular casts which indicate acute damage to the nephron After the passage of haemoglobin has ceased the casts rapidly disappear but the protein may persist in the urine in progressively greater dilution for some days Haemoglobinuria is by no means always accompanied by changes in urinary flow The latter seem to be in fact independent of the passage of haemoglobin since oliguria and anuria develop sometimes when the urine is clear of blood pigments and fail to appear when there is massive haemoglobinuria even in the presence of an acid urine Leaving aside for the moment the question of the part played by haemoglobin and its derivatives in the development of the renal failure in blackwater fever it can be seen that tubular damage is a prominent feature of the disease as indicated by the passage of tubular casts and sometimes occasional erythrocytes Thus tubular damage is present whether or not changes in urinary flow develop It appears sometimes to be exacerbated during the passage of haemoglobin but the degree of damage is not very closely related to the degree of haemoglobinuria In mild cases the diminution of urinary flow is limited and the oliguria which may last for several days and be accompanied by a rising blood urea and sometimes oedema is eventually succeeded by polyuria and recovery In the more severe cases there may be practically complete suppression of urinary flow and the patient passes into a state of acute progressive uraemia Even in the most severe cases however recovery of urinary flow occasionally takes place and is followed by a post anuric period of polyuria associated with the passage of dilute urine

Patients in whom the urinary output is unaffected show few signs of renal disturbance beyond the presence of albumin casts and sometimes erythrocytes in the urine In oliguric cases the blood urea nitrogen may rise considerably and some generalized oedema may develop The classical picture of renal failure is seen as a rule only in the anuric case Here the urine that is passed and usually an ounce or two is secreted in 24 hours may or may not contain haemoglobin and may be acid neutral or alkaline It is usually heavily loaded with albumin tubular casts are abundant and the urea and chloride concentrations are low The urinary flow may cease abruptly or there may be a

commonest in *P falciparum* infections They vary from simple albuminuria with casts and blood cells in the urine to syndromes which resemble nephritis or nephrosis The latter syndromes appear in two forms The commonest is a hydraemic nephrosis with some degree of oliguria general oedema and signs of water retention More rarely there may be a picture closely simulating the acute nephritis of streptococcal infections with severe oliguria nitrogen retention and uraemia Occasionally in recurrent malaria (usually in *P malariae* infections) exacerbations of the infection may be accompanied by acute exaggeration of existing renal symptoms leading to renal failure and death in uraemia

It is not clear why the acute syndromes of renal failure should appear most frequently in P *falciparum* infections Giglioli (1930) has pointed out that this infection has less tendency to chronicity and relapse than the others and that it acts more vigorously so that any specific action on the tissues e g on the renal epithelium would be concentrated over a short period of time Giglioli was thinking in terms of specific toxins but his remarks apply equally well to the circulatory and other changes of a general nature which occur in malaria and which are most active and concentrated in *P falciparum* infections These points will be discussed later It must however be noted here that the presence of a heavy infection with parasites is not necessary in the development of renal failure in malaria Local damage to the kidney tissue is seldom directly due to parasitic accumulations In fact the most serious pathological changes arise in a condition in which parasites are commonly scanty namely blackwater fever

Renal failure is the commonest complication of blackwater fever It is probably fair to say that such failure in blackwater fever is partly derived from other processes besides the malarial infection but in many ways the development of renal damage in this disease illustrates the mechanisms involved in producing kidney damage in malaria uncomplicated by excessive haemolysis and haemoglobinuria The element of renal tubular damage is common to the early stages of both conditions and probably has a common pathogenesis In blackwater fever however the renal tubular damage is often exaggerated by an almost complete suppression of urinary secretion associated with nitrogen retention The patient dies in a state of acute uraemia similar to that arising in many other conditions such as cholera and incompatible transfusion In malaria as we have seen the syndrome more frequently goes on to a much milder hydraemic nephrosis

Renal failure which accounts for half the deaths in blackwater fever

tions who developed marked oedema and signs of diffuse nephritis These cases had suffered from untreated malaria for some time

Many observations of a similar kind have been made in more recent years and the syndrome of oedema and renal symptoms appearing during the course of chronic or recurrent malaria is now well authenticated The syndrome as was indicated in a previous section of this Chapter is now thought to be in the nature of a nephrosis rather than a nephritis

Macfie and Ingram (1917) in the Gold Coast concluded from their review of the literature that nephritis developed most often in malaria which had been untreated or badly treated or in subjects who had had repeated attacks They reported nine cases in which renal symptoms were well established All were children under 10 years of age Some were suffering from mixed infections but all had quartan parasites in the peripheral blood The renal symptoms developed gradually with progressive oedema of the face hands and feet three patients had ascites The urine was examined in five cases only albumin was present in all cases but casts are mentioned in only one no information on this point being provided in the other four cases Adequate quinine therapy resolved the renal symptoms in all cases

Clarke (191- 19-9) reported that more than 50 per cent of cases diagnosed as nephritis in Perak had *P malariae* in their blood Of 62 cases showing oedema and albuminuria 3- had parasites and only five had fever These cases responded well to quinine therapy

Manson-Bahr and Maybury (19-7) described two cases of nephritis both associated with *P malariae* infection One case showed oedema of the feet and face and the urine contained 1 5 per cent albumin and granular and hyaline casts Response to quinine therapy was slow The second patient first developed quartan malaria a year before the onset of the oedema which first appeared three months after a recurrence of the infection The swelling began in the ankles and by the time of examination involved the face legs arms abdominal wall and sacroiliac region The urine contained 1 per cent albumin epithelial cells hyaline and granular casts and red blood corpuscles The blood pressure was 145 90 and there was some cardiac hypertrophy The case therefore suggested some evidence of azotemic nephritis There was a rapid response to quinine one month after treatment the oedema had subsided and the systolic blood pressure was 130 mm Hg

Giglioli (1930) made a thorough survey of the incidence of nephritis and its relation to malaria in British Guiana between the years 1923 and 1929 There was a malaria epidemic in 1926 Nephritis became

short period of oliguria before the suppression develops The blood urea concentration which is raised above normal in most blackwater fever cases (Fairley and Bromfield 1934 Maegraith 1944) is often high before the onset of oliguria or anuria once renal failure develops it rises very rapidly and may reach very high levels In recovery after anuria it remains elevated for some days and returns slowly to normal in the course of two or three weeks There is usually a pronounced fall in plasma chloride during the anuric phase the concentration slowly returning to normal in recovery

An abrupt fall in blood pressure sometimes immediately precedes the onset of anuria but as the renal failure progresses the blood pressure tends to rise (Maegraith 1944) There is however frequently a rapid fall of blood pressure particularly the diastolic pressure in the late stages of the anuric state and towards the end the patient commonly passes into a state of medical shock complicated in some cases by motor hyperactivity and vomiting

In blackwater fever there may thus be two clinical forms of renal impairment In one there are albumin and casts in the urine and mild symptoms of tubular epithelial dysfunction In the other there is added to the first syndrome the complication of urinary suppression which may go on to complete anuria and extreme exacerbation of the tubular failure Recovery in the latter case is associated with polyuria and the passage of dilute urine a condition also seen after recovery from malarial nephritis (Craig 1909) The reversible nature of the anuria syndrome is the feature which distinguishes it from other forms of acute nephritis As will be seen later it may be classified as an example of the syndrome of renal anoxia (Maegraith *et al* 1945)

RENAL DYSFUNCTION IN CHRONIC AND RECURRENT MALARIA

Watson (1905) in reviewing the clinical features of natural *P malariae* infections reported that in 83 cases 27 individuals showed various degrees of oedema ranging from slight swelling of the feet to oedema of the hands and arms legs abdomen chest and face In severe cases there was sometimes pleural effusion and some pulmonary oedema The urine usually contained albumin and casts sometimes in large quantities Fever was frequently but not always present and the condition responded well to adequate antimalarial treatment James (1910) described two patients suffering from *P malariae* infec-

depending on the presenting symptoms The first group complained of long drawn out fever and had no obvious signs of renal dysfunction except for occasional swelling of the ankles and face The urine volume was reduced the urine of high specific gravity and usually acid It contained albumin and abundant hyaline and granular casts but no red cells Urea concentration tests usually demonstrated impairment of concentration and the diastatic test impaired permeability The blood pressure was normal Under adequate antimalarial treatment the signs and symptoms were relieved Many of these cases returned subsequently to hospital with well established renal syndromes

In the second group of patients corresponding to what Giglioli calls chronic parenchymatous nephritis the malaria was usually in an active clinical state on admission The individual complained chiefly of generalized oedema the onset of which was sudden and related to a recent recrudescence of malaria or even the presenting attack The swelling involved the face abdominal wall genitals and legs There was often ascites The urine was scanty and contained large amounts of albumin The specific gravity was low and there was a sediment containing granular and hyaline casts and occasionally red corpuscles Urea concentration and diastatic tests gave results much below normal Both *P vivax* and *P malariae* were found in the blood

The third group of patients had less marked but more persistent oedema headache vomiting There was less albumin in the urine and fewer casts than in the second group The volume of urine secreted daily was high The blood pressure was raised and signs of cardiovascular alterations were present In these cases the onset was very gradual the history being one of low grade fever which was so mild that treatment was not thought necessary *P malariae* was the commonest parasite observed in the blood in this group which Giglioli classifies as the chronic interstitial type of nephritis

Giglioli stated that once definite nephritis had been established the condition unless energetically treated progresses with exacerbations corresponding to those of the underlying malaria infection until death supervenes in a relapse with symptoms of acute renal failure

Surbek (1931 a) described a syndrome very similar to that described by Giglioli occurring in patients in Sumatra He called the condition *quartana nephrosis infantum ac adolescentium* and described it as a subacute parenchymatous hydropic nephritis with oedema frequent ascites and heavy albuminuria and hyaline and pigmented casts but usually no erythrocytes in the urine It occurred in children and

especially prominent during the post-epidemic period in the succeeding two years when chronic infections abounded Seasonal rises in the incidence of malaria in these years was associated with 'exacerbations of the renal syndromes In 87 cases of nephritis examined malaria parasites were found in 61 He therefore concluded that malaria was the significant aetiological factor in the development of the endemic nephritis of the area in which he was making his observations Seventeen cases were infected with *P malariae* only and two had a mixed infection with *P malariae* and *P vivax* There were only 35 cases of quartan malaria so that the incidence of the renal syndrome in this infection was nearly 50 per cent Just over 4 per cent of the *P vivax* cases showed renal symptoms In the indigenous population children were most frequently affected

Giglioli did not observe any acute nephritis The 102 cases he examined were all either subchronic or chronic dropsical or interstitial nephritis All cases gave a clinical history of chronic relapsing nearly continuous fever The majority had acquired malaria during the local epidemic of 19-6 and the infections had subsequently been treated inadequately The malarial attacks were notable for their persistence and tendency to frequent relapse rather than their initial severity

Giglioli considers that the sequence of events in the evolution of the renal syndrome in malaria is as follows Albuminuria in acute cases represents an element of transitory and reversible renal damage which disappears with the other symptoms when the clinical activity of the malaria subsides In persistent chronic and relapsing infections albuminuria becomes permanent and gradually increases Casts and cells are eventually passed in the urine and at a later stage oedema appears and nephritis representing severe renal damage has become established In the early stages the condition is reversible and will respond to treatment but if the disease is allowed to progress unchecked permanent kidney damage occurs in the form of the complex symptomatology of chronic parenchymatous or chronic interstitial nephritis The late incidence of the renal syndrome in relation to the epidemic of 19-6 and the fact that it was found in 19-8-9 in children of two or three years of age but not younger indicated the slow progress of the condition

Giglioli concluded that uncomplicated albuminuria and established chronic nephritis were merely different phases of the same slow process of kidney damage brought about by low grade chronic malarial activity

He found that his patients could be divided into three groups

the nephritis developed during a clinically active attack of malaria. In one case a man aged [illegible]1 the fever was subdued immediately by quinine therapy but the patient died two days after admission. At autopsy the kidney showed the lesions of chronic nephritis and there was hypertrophy of the left side of the heart.

Carothers (1934) in East Africa reported 15 cases of subacute nephritis in children from 3 to 1[illegible] years of age. Three cases were fatal. The syndrome was one of marked general oedema especially of the legs and face. Twelve children had ascites. The urine in all cases contained albumin. Ten of the 15 subjects were infected with *P malariae* compared with 18 out of [illegible]17 other children suffering from malaria. The rate of infection with *P falciparum* was the same in nephritic and other children.

James (1939) studied malarial nephrosis in New Guinea and the Solomon Islands. He reported [illegible] cases in 10 years. Nine were infected with *P malariae* two with *P malariae* and *P vivax* two with *P vivax* and *P falciparum* and two with unidentified parasites. The clinical picture was very similar to that described by Giglioli and Surbek. Oedema was general and was particularly evident in the face legs and scrotum. Ascites was common. There was no increase in blood pressure and no change in blood urea nitrogen. The urine contained albumin in half the cases associated with granular and cellular casts renal cells leucocytes and often scanty erythrocytes. The cases were all chronic malarial subjects with some degree of anaemia and splenic enlargement. The syndrome occurred most commonly in children 17 being under seven years of age. In most cases there was a long history of chronic or recurrent malaria and a relatively short history of renal involvement. Adequate treatment of the malaria with quinine was usually accompanied by recovery or partial recovery from the renal symptoms. In a few cases oral quinine had no effect and the patients died.

There have been many other accounts of chronic malarial nephritis or nephrosis developing in the course of naturally acquired infections most of them describing a clinical picture similar to that outlined above. The features that have been particularly stressed are the incidence of the syndrome in *P malariae* infections in young people and the often dramatic response to quinine therapy (Heilig 1941 Leading Article *Indian Medical Gazette* 194[illegible]).

In induced malaria the fully developed syndrome does not seem to have been reported but there are numerous accounts of albuminuria

adolescents infected with *P malariae* and in its early stages responded well to quinine In a subsequent paper (1931 b) he gave a more detailed account of the syndrome in a young Javanese man who had had severe quartan malaria (untreated) for two months before admission and who died three days later in spite of quinine therapy *P malariae* parasites were present in the peripheral blood There was diffuse oedema of the nephrotic type and very marked ascites The patient was prostrated vomited and had persistent diarrhoea. Apart from drowsiness there was no evident uraemia but there was an azotemia of 1 per cent (later corrected to 1 per mille) The erythrocyte count was 2 9 million cells per cu mm. and there was an intense infection with *P malariae* There was heavy albuminuria and the urine contained a deposit in which there were abundant casts of every description and a few erythrocytes The urinary chloride concentration was low Treatment with quinine reduced the number of parasites in the blood but the patient died apparently in uraemia. Surbek refers to 10 cases of this renal syndrome seen in Sumatra two of which were fatal All occurred in subjects under 30 years of age and seven in children.

Goldie (1930) reviewed the literature and came to the conclusion that any form of malarial infection if allowed through lack of treatment or inadequate treatment to become chronic or recurrent might lead to the appearance of chronic nephritis of the hydraemic type Goldie and his colleagues examined 674 cases of benign tertian, 381 of malignant tertian and 23 of quartan malaria Four cases of quartan one case of benign tertian and three cases of malignant tertian malaria showed oedema and three of the quartan cases gave evidence of water retention. Water retention was also observed in 10 cases of malignant tertian and two of benign tertian malaria Renal complications were thus commonest in quartan malaria They considered that this was probably because of the tendency of this infection to become chronic or latent The symptoms of nephritis in malaria were usually albuminuria local or generalized oedema and water retention i e the features of hydraemic nephritis Cardiovascular symptoms related to the azotaemic form of nephritis occurred in a few cases

Jansco and d Engel (1931) described similar renal syndromes in 14 cases of malaria in Hungary 10 of which were *P falciparum* infections three *P malariae* and one mixed *P vivax* and *P malariae* The clinical picture was that of chronic nephritis even in the *P falciparum* infections The malaria infection responded well to quinine but the symptoms of nephritis were unaffected In the *P malariae* infections

the nephritis developed during a clinically active attack of malaria. In one case, a man aged 21, the fever was subdued immediately by quinine therapy, but the patient died two days after admission. At autopsy the kidney showed the lesions of chronic nephritis and there was hypertrophy of the left side of the heart.

Carothers (1934) in East Africa reported 15 cases of subacute nephritis in children from 3 to 12 years of age. Three cases were fatal. The syndrome was one of marked general oedema, especially of the legs and face. Twelve children had ascites. The urine in all cases contained albumin. Ten of the 15 subjects were infected with *P. malariae* compared with 18 out of 217 other children suffering from malaria. The rate of infection with *P. falciparum* was the same in nephritic and other children.

James (1939) studied malarial nephrosis in New Guinea and the Solomon Islands. He reported 22 cases in 10 years. Nine were infected with *P. malariae*, two with *P. malariae* and *P. vivax*, two with *P. vivax* and *P. falciparum*, and two with unidentified parasites. The clinical picture was very similar to that described by Giglioli and Surbek. Oedema was general and was particularly evident in the face, legs and scrotum. Ascites was common. There was no increase in blood pressure and no change in blood urea nitrogen. The urine contained albumin in half the cases, associated with granular and cellular casts, renal cells, leucocytes and often scanty erythrocytes. The cases were all chronic malarial subjects with some degree of anaemia and splenic enlargement. The syndrome occurred most commonly in children, 17 being under seven years of age. In most cases there was a long history of chronic or recurrent malaria and a relatively short history of renal involvement. Adequate treatment of the malaria with quinine was usually accompanied by recovery or partial recovery from the renal symptoms. In a few cases oral quinine had no effect and the patients died.

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In induced malaria the fully developed syndrome does not seem to have been reported but there are numerous accounts of albuminuria

urinary casts and sometimes oedema associated particularly with *P malariae* infections (Boyd and Proske 1941 Kitchen 1941)

A somewhat unusual syndrome has recently been described by Sarrouy and Portier (1939) It occurred in a young Arab woman with a history of fever and oedema developing a month before admission to hospital and a similar attack 12 months previously *P malariae* rosettes were present in the blood She was suffering from generalized oedema and the urine contained considerable quantities of albumin. There were however neither casts nor blood cells in the urine and the urinary urea output was normal The blood pressure was not raised but the blood urea nitrogen was 77 mgm per cent There was an increase in total blood lipoid and cholesterol a fall in albumin and a rise in haemoglobin concentration The blood chloride remained normal even during the most severe oedema Antimalarial treatment combined with liver therapy had a good effect The authors called this condition nephrose lipoidique

At first sight there appears to be wild confusion amongst authors with regard to the description and classification of the various renal syndromes they describe in chronic or recurrent malaria but the confusion lies more with the classification than the description Giglioli s three groups of syndromes with a few modifications cover most of the cases described Recapitulated these are

(*a*) A slowly developing albuminuria with casts in the urine

(*b*) Generalized oedema of varying degree with oliguria albuminuria casts and sometimes red blood cells in the urine no rise in blood pressure or blood urea nitrogen uraemia only in the final stages

(*c*) Some oedema some albuminuria and casts and occasionally red blood cells in the urine no oliguria except in the final stages a rise of blood pressure and blood urea nitrogen sometimes cardiac hypertrophy uraemia a common outcome

Giglioli calls the last two syndromes chronic parenchymatous nephritis and chronic interstitial nephritis respectively Surbek calls the second syndrome *quartana-nephrosis infantium ac adolescentium* Goldie differentiates the two syndromes as hydraemic and azotaemic nephritis The following labels have also been used diffuse nephritis dropsical or interstitial nephritis parenchymatous hydropic nephritis subacute nephritis nephrosis and nephrose lipoidique the latter referring to the case described by Sarrouy and Portier which is somewhat unique in that there appeared to be a fatty nephrotic syndrome in which the renal function was unimpaired

The true nature of these syndromes cannot be determined without an account of the pathological changes and the functional deviations associated with them There is ample information regarding the pathological changes but very little concerning the kidney function during the syndrome

Where investigation has been carried out however it has been found that renal function is frequently impaired. For instance Wolsky and Schewelewa (1930) investigated renal function using the methods of Volhard and of Mosenthal in 76 cases of malaria at all stages including the quiescent period between relapses Of 34 cases showing signs of renal dysfunction 15 were vivax malaria (out of 23 cases) six were falciparum (out of 10 cases) and 13 were quartan (out of 19) The authors concluded that renal dysfunction occurred with equal frequency in the three forms of malaria They also obtained evidence indicating a relation between the appearance of renal dysfunction in malaria and the degree of enlargement of the spleen

Toscano (1931) examined renal function in 1[illegible] cases of malaria and found a diminution in the dilution power of the kidney in seven (six falciparum and one vivax malaria) Interference with concentration of urea was evident in four cases (three falciparum one vivax) In the only two cases in which tests were repeated after specific therapy normal function was restored He could find no relation between the gravity of the general symptoms and the appearance of kidney dysfunction

The combined functional and pathological evidence supports the view that in malaria the emphasis is on tubular damage of a degenerative rather than inflammatory nature so that the syndrome on the whole is of the nature of a nephrosis rather than a nephritis

PATHOLOGICAL CHANGES

In the following section the pathological changes in the kidneys in malaria are subdivided for the purpose of discussion into (i) those of a general nature arising directly from the parasitic invasion and (ii) those more specifically related to the parenchymatous tissue The lesions in blackwater fever are treated separately

(1) Changes arising directly from malarial infection

Parasites

Parasites are usually scanty in the kidneys even when they are plentiful in other organs Craig (1909) considered that they occurred in numbers that were too small to account for the development of the renal lesions They are unevenly scattered through the organ and are found most frequently in the intertubular capillaries and less commonly in the glomerular vessels They are rarely found in the renal veins (Craig 1909 Deaderick 1909 Marchiafava and Bignami 1900 Gaskell and Millar 1920)

Occasionally the kidney vessels may be filled with parasitized cells to the point of apparently obstructing the blood flow Such a case has been reported by Ewing (1901) At the other extreme Allen (19 6) has described four cases of acute *P falciparum* infections in which there were gross renal pathological changes but no parasites were found in the kidneys although they were abundant in other organs

Pigmentation and phagocytosis

The kidneys are not commonly heavily pigmented but haemozoin is frequently found in small amounts in the glomerular tufts in the intertubular vessels sometimes free in the interstitial tissue and even occasionally in the epithelial cells of the tubules and within phagocytes in the capsular space The glomeruli usually contain more pigment than the other tissues Very rarely pigmentation may be general and extreme and the intertubular vessels may become marked out by haemozoin within phagocytes contained in the vessels and according to some authors in the vascular endothelium Pigment giving the Prussian blue reaction for iron is often scattered sparsely over the whole organ including the glomerular tufts and tubular epithelium

Phagocytosis of erythrocytes parasitized erythrocytes and pigment is not pronounced in the kidney although the pigment present is mostly intracellular In the glomeruli pigment has been reported in both epithelial and endothelial cells (Craig 1909 Marchiafava and Bignami 1900) and in perivascular cells on the capillaries (Torrioli 1932) As mentioned above pigment has also been reported in the endothelial cells of the intertubular capillaries Allen (19_6) found malarial pigment coarse and fine in mononuclear cells which collected in the interstitial tissue in areas in which the degenerative changes in the tubular epithelium were not very advanced' Taliaferro and Mulligan (1937) found little evidence of phagocytosis in simian malaria in either

the acute or chronic stages of the disease Pigment was occasionally present in macrophages lying in the interstitial tissue and in the late stages of acute *P knowlesi* infections pigmented monocytes were found in the capillaries together with unphagocytosed parasitized erythrocytes

Vascular changes

Scattered small haemorrhages in the cortex and medulla have been described especially in acute renal syndromes Mild and severe fatty and mild degenerative changes in the endothelium have been noted especially in the glomerular tufts and afferent arterioles Gross swelling or proliferation of the endothelium has not been described Thrombosis stasis and intravascular agglutination of erythrocytes have been described only in relation to lesions in cases in which there is heavy parasitaemia involving the renal vessels Occasionally infarcts have been reported In chronic and recurrent *P malariae* malaria Giglioli (1932) has described thickening of the arteries and arterioles of the kidney associated with cardiac hypertrophy In these cases the fatty degenerative changes in the glomerular capillaries were most marked.

(ii) Changes in the parenchyma

A Acute malaria

In acute malarial infections in which there has not been marked clinical evidence of renal involvement there are usually few pathological changes in the kidneys The organs are slightly enlarged and not deeply pigmented The capsule is non adherent and strips easily The vessels of both cortex and medulla may be congested on the other hand the kidneys may be pale There are sometimes punctate haemorrhages in the *mucosa* of the pelvis The cortex is frequently slightly thickened Microscopically the lesions peculiar to malaria are much less in evidence than in other organs (Craig 1909) The glomerular tufts are sometimes congested and in severe cases may be the site of small haemorrhages There may be some slight proliferation of the epithelium of Bowman s capsule and the capsular space sometimes contains fibrinous or albuminous matter and even pigmented leucocytes and plasmodia (Craig) There is cloudy swelling of the epithelium of the tubules which in severe cases may exhibit fatty degeneration and necrosis The tubules especially the straight tubules (Deaderick 1909) contain hyaline epithelial and granular casts The intertubular vessels are often congested and contain parasitized red cells and pigmented phagocytes

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Parasites

Parasites are usually scanty in the kidneys even when they are plentiful in other organs Craig (1909) considered that they occurred in numbers that were too small to account for the development of the renal lesions They are unevenly scattered through the organ and are found most frequently in the intertubular capillaries and less commonly in the glomerular vessels They are rarely found in the renal veins (Craig 1909 Deaderick 1909 Marchiafava and Bignami 1900 Gaskell and Millar 19-0)

Occasionally the kidney vessels may be filled with parasitized cells to the point of apparently obstructing the blood flow Such a case has been reported by Ewing (1901) At the other extreme Allen (19-6) has described four cases of acute *P falciparum* infections in which there were gross renal pathological changes but no parasites were found in the kidneys although they were abundant in other organs

Pigmentation and phagocytosis

The kidneys are not commonly heavily pigmented but haemozoin is frequently found in small amounts in the glomerular tufts in the intertubular vessels sometimes free in the interstitial tissue and even occasionally in the epithelial cells of the tubules and within phagocytes in the capsular space The glomeruli usually contain more pigment than the other tissues Very rarely pigmentation may be general and extreme and the intertubular vessels may become marked out by haemozoin within phagocytes contained in the vessels and according to some authors in the vascular endothelium Pigment giving the Prussian blue reaction for iron is often scattered sparsely over the whole organ including the glomerular tufts and tubular epithelium

Phagocytosis of erythrocytes parasitized erythrocytes and pigment is not pronounced in the kidney although the pigment present is mostly intracellular In the glomeruli pigment has been reported in both epithelial and endothelial cells (Craig 1909 Marchiafava and Bignami 1900) and in perivascular cells on the capillaries (Torrioli 1932) As mentioned above pigment has also been reported in the endothelial cells of the intertubular capillaries Allen (1926) found malarial pigment coarse and fine in mononuclear cells which collected in the interstitial tissue in areas in which the degenerative changes in the tubular epithelium were not very advanced' Taliaferro and Mulligan (1937) found little evidence of phagocytosis in simian malaria in either

cases of *P falciparum* infection in which in his view the renal damage was the cause of death In these cases the kidneys were considerably enlarged the capsule was non adherent and the organ surface was smooth and reddish grey The substance was firm The cut surface was greasy and the cortex could not easily be distinguished from the medulla and the medullary rays were indistinct

The outstanding histological feature was the advanced degeneration of the epithelial cells of the tubules In large areas these cells were completely degenerated the cytoplasm granular and the nuclei broken up The lumina of the tubules were frequently filled with granular debris The interstitial tissue between the tubules contained numbers of large pigmented mononuclear cells which were present in greatest numbers in areas where the cells were least degenerate The glomerular tufts contained malarial pigment and there was some desquamation of the epithelium of Bowman s capsule As mentioned above no parasites were found in the kidneys in these cases although they were present in other organs

Allen stated that the general histological picture was not unlike that described in the later stages of blackwater fever where some observers lay stress on the frequency with which degenerative changes are seen in the cells lining the tubules

Allen s description corresponds closely with that of most other workers Goldie (1930) reviewed the literature and came to the



F 10—M l gn nt ter ı al n noa n S t n thr ghr l m d lla N t t b la ch ges

Sometimes there are capillary haemorrhages and signs of pressure necrosis (Craig 1909 Marchiafava and Bignami 1900 Thayer 1899 Deaderick 1909 Dudgeon and Clarke 1917)

Gaskell and Millar (1920) have described these changes very clearly in cases which died without overt renal symptoms from *P falciparum* infections in Salonika during the 1914–18 war In cerebral cases the kidneys were not greatly enlarged the capsule stripped readily the cortex was not markedly swollen and the cut edges did not evert The cortical blood vessels were distended with blood The glomerular endothelial cells showed irregular mild fatty degeneration and the capillaries were congested The cells of the proximal convoluted tubules were swollen and their inner margins irregular There was some loss of nuclear staining The large vessels at the junction of the cortex and medulla were congested as were the medullary capillaries The endothelial lining of the latter displayed some mild fatty degenerative changes The tubules other than the convoluted tubules were apparently normal There were few parasites and little pigment In septicaemic cases in which the general degree of parasitaemia was very great parasites were not present in the kidneys in large numbers and the renal changes were similar to the above except for occasional haemorrhages in the subcapsular regions and in the mucosa of the pelvis (The latter was also described by Marchiafava and Bignami) In the cardiac type of case in which cardiovascular symptoms predominated parasites were also scanty in the kidneys The cortex was swollen and pale the vessels dilated and congested The glomeruli were normal except for occasional parasites or pigment There was however appreciable degeneration of the epithelial cells of the proximal convoluted tubules in which nuclear staining was poor and there were pronounced granular changes in the cytoplasm The cells of the distal convoluted tubules had in places desquamated and filled the tubule lumen with debris The medullary portions of the tubules were not affected The medullary vessels were irregularly congested the congestion being especially pronounced in certain areas

It is clear from the above that the changes in the kidney occurring in cases of malaria in which the clinical signs of severe renal disturbances have not developed to the stage of a nephrosis or nephritis are chiefly to be found in the tubules The glomeruli apart from some pigmentation and congestion are not much affected In cases of so-called acute malarial nephritis or nephrosis this concentration of damage on the tubules is even more clearly marked

Severe lesions have been described by Allen (1926) in Jamaica in four

d velopment of severe oliguria or anuria however similar lesions are apparently capable of giving rise to an azotaemic clinical picture with rising blood pressure and blood urea As will be seen later there is evidence to show that this azotaemia is the outcome of the failure of glomerular filtration consequent on failure of glomerular blood flow and not of the tubular changes

Glomerular changes may occur in acute malaria uncomplicated by other diseases but they are seldom severe and are rarely as pronounced as the changes in the tubules

B Chronic and recurrent malaria

Goldie (1930) reviewed the literature to that date and pointed out that descriptions of kidney changes in chronic or recurrent malaria varied from big fat kidney (Kelch and Kiener) to small granular kidney (S van) Volhard and Fahr considered that the essential lesion was a diffuse fatty degeneration associated in acute cases with epithelial necrosis Munk called the lesion a nephrosis and stated that signs of inflammation of the renal tissue seldom accompanied it even when the kidney vessels were loaded with parasites Ewing s (1901) findings in a single case were similar but Giglioli (1932) found considerable inflammatory or cellular changes in three of five cases of quartan nephrosis Goldie concluded from his survey that fatty changes and degeneration of the tubules were the commonest findings the pathological picture being that of a nephrosis of the hydraemic type In acute malaria this nephrosis occurred mainly in *P falciparum* infections but in chronic or recurrent malaria it was commoner in *P vivax* and *P malariae* infections The differences between the pathological chang s in the former infection and in the latter depended upon the more toxic activity of *P falciparum* and the tendency for the other species to relapse and become latent

In gen ral the descriptions of kidneys given by workers in this field fall into two groups In one the kidney is large tense and yellow-white or grey and the essential lesion is tubular epithelial degeneration with few if any changes in the glomeruli In the other the kidneys are not enlarged or are contracted and there are changes in the glomeruli of varying degree with massive and irregular infiltration of the tissue with round cells associated with an active hyperplasia of connective tissue Giglioli has suggested that these two lesions might be related the second succeeding the first He has suggested that the evolution of the renal lesions may develop as follows in acute *P falciparum* and occasionally *P malariae* infections the kidney condition is either cured

conclusion that the principal renal lesions in malaria were fatty and other forms of degeneration of the tubular epithelium Nevertheless



FIG 11—Crush inj y anu ia Sectio thr gh enal med lla Note tub l changes and conte ts

glomerular changes have been occasionally reported in acute malaria Marchiafava and Bignami (1900) for instance are often quoted as describing a glomerulo-nephritis in a case of quartan malaria In this case however as in some of the others quoted (Jansco and d Engel 1931) there was the complicating factor of a secondary infection and it is impossible to separate the malarial from the other elements in the genesis of the kidney syndrome and pathological picture Giglioli (1932) has described advanced glomerular changes in *P malariae* infections but only in chronic or recurrent malaria and also in some cases in the presence of secondary infections

Spitz (1946) found enlarged ischaemic and cellular glomeruli and swelling of the tuft endothelium in nine of a series of 50 cases of malignant tertian malaria These cases had had azotaemia and four also had what she described as hemoglobinuric nephrosis similar to that seen in blackwater fever and incompatible transfusion Changes in the tubular epithelium were noted in the majority of cases

It appears therefore that the characteristic kidney lesion of acute malaria is degeneration of the tubular epithelial cells This as Goldie points out is the pathological pattern of a nephrosis rather than a nephritis and is accompanied usually by a hydraemic clinical picture Under certain conditions especially in circumstances leading to the

with necrosis in some areas These changes were most pronounced in the proximal convoluted tubules where the epithelium was shed and appeared in granular masses with clumps of broken-down nuclei forming epithelial casts The fatty change in the epithelial cells was most pronounced near the basement membrane Granules of haemozoin were present in the epithelial cells of the proximal convoluted tubules The capillaries associated with the tubules showed no evidence of embolic blockage with pigment The glomeruli showed fatty degeneration of the epithelial lining and some fatty changes in the capillary endothelial cells These changes were not as pronounced as those seen in the tubules Parasites of unidentified species containing pigment were present in the capillaries There was some swelling of the epithelium and basement membrane in Bowman s capsule Compared with the changes in the tubules the glomerular changes were very slight The blood vessels were unchanged

Other authors have described similar lesions in cases of malaria usually in quartan but occasionally even in malignant tertian malaria (Jansco and d Engel 1931) The prominent lesion is degeneration of the tubular epithelium usually most pronounced in the proximal convoluted tubules associated with desquamation of the epithelial debris into the lumen The glomerular changes are slight and are seldom more severe than a mild fatty degeneration of the epithelial lining membrane and the endothelial cells of the capillaries Sometimes as in Surbek s case round cell infiltration of the interstitial tissue especially in the medulla is mentioned This is usually not pronounced but in a group of cases described by Giglioli it appeared as a prominent feature and was associated with proliferation of the interstitial connective tissue The clinical histories of Giglioli s patients were all long both from the point of view of malaria and renal symptoms so that it is possible that the cases represent a more advanced stage of the process which gives rise to the lesions already described Giglioli s five cases can be divided into two in which the kidneys were enlarged and tense and in which the capsule was non-adherent and two in which the kidneys were smaller than normal and in which the capsules were adherent One case was complicated by gangrene of the pudenda

Case I belonged to the first group The patient was a child aged two years and a half at death She had been infected with *P malariae* for a year and had had an attack of oedema accompanied by albuminuria and urinary casts successfully treated with quinine six months before the final similar attack of oedema and proteinuria which failed

or if untreated proves fatal Occasionally however it may progress into more chronic state In *P vivax* and *P malariae* infections the process is more gradual and a sub-chronic nephritis develops and is succeeded in due course by a chronic parenchymatous nephritis and ultimately by chronic interstitial nephritis In the initial stages the changes are reversible and give rise clinically to albuminuria and the passage of casts later the symptoms of hydraemic nephrosis supervene and finally may give place to those of terminal uraemia There is no more than presumptive evidence that the kidney lesions progress in this way but the history of individual patients is often strongly suggestive and it is convenient here to adopt such a hypothesis in order to simplify the presentation of the evidence

The large white kidney type of picture is well illustrated in the case described by Surbek (1931) The patient was a Javanese who had had oedema and ascites for two months before admission His blood showed a heavy infection of *P malariae* and although quinine relieved the malaria he died three days later from uraemia The kidneys were greatly enlarged and pale white in colour The cut surface was opaque and the capsules were non-adherent The cortex showed degenerate convoluted tubules the cells showing small drops and granulations which were light rose in colour The lumina were filled with desquamated cells and coagulated masses of the same colour and intensely coloured hyaline casts The epithelial cells also showed fatty degeneration Some fatty degeneration of the glomerular cells was also observed otherwise the glomeruli were unchanged A few capsules contained debris similar to that in the tubules The cortical blood vessels were unchanged The medullary portions of the tubules contained hyaline casts and there were scattered patches of round cell infiltration Surbek described the picture as degenerative parenchymatous nephritis the slight glomerular changes being outweighed by the alterations in the convoluted tubules

A similar picture was described by Menon and Annamalai (1933) in a Hindu woman with oedema of the legs who was found comatose and moribund The kidneys were both pale greyish white tense and much enlarged The capsule was non-adherent The cut surface bulged and bled very little Cortex and medulla were both swollen and pale and the demarcation was not clearly defined The authors describe the appearance as being somewhat similar to the large white kidney of subacute nephritis The glomeruli were not visible but there was some congestion of the papillae of the pyramids The tubular epithelium showed advanced cloudy swelling and fatty degeneration

of the glomeruli than of the tubules the lesions of which he believed to be largely secondary to the changes in the interstitial connective tissue and glomeruli On this basis he regarded them as examples of chronic glomerular nephritis in the active and terminal stages of development In his view the evidence indicated that in the early stages of this chronic disease in *P malariae* infections the renal lesions were primarily degenerative whereas in the later stages they were principally inflammatory and proliferative

There is general agreement amongst workers on the appearance of degenerative lesions but few have recorded the diffuse cellular infiltrative and proliferative picture or the severe glomerular changes described by Giglioli It is possible that some of the lesions described by the latter may have arisen as in his second case which died of gangrene of the pudenda from factors other than the malarial infection

The kidney in blackwater fever

Macroscopic appearances

Descriptions of the kidneys in blackwater fever vary considerably There appear to be few specific appearances even in anuric cases although all workers agree that the organs are enlarged and many report some degree of oedema and tenseness of the tissues Some authors have found the kidneys generally engorged and irregularly marked with haemorrhages which are mainly concentrated in the cortex Some have reported that the congestion of vessels is chiefly confined to the cortex others have observed the main vascular changes in the medulla (Ameuille *et al* 1918 Dudgeon 1920 Laterni 1923 1928) A relative anaemia of the cortex associated with congestion of the medulla has frequently been noted particularly in anuric cases and has been referred to as the essential lesion For example Pellarin (1876) described the cortex in one case as pale grey and the pyramids much congested The cortex has often been described as stippled with brown spots and the medulla dark brown with radiating brown lines (Barratt and Yorke 1909 Thomson 1924 Rapaport 1928 Stephens 1937)

In the cases seen in West Africa during the 1939–45 war the appearance of the kidneys was fairly uniform The organs were enlarged swollen and slightly oedematous They were pale or light brown depending upon the amount of pigmented material present in the tubules and general congestion was seldom obvious In relation

to respond to quinine The kidneys were enlarged the capsule was non adherent and the underlying surface was smooth and greyish yellow The cortico-medullary ratio was normal the cortical pattern obscured and the pyramids congested at their bases Histologically the glomeruli were practically bloodless the nuclei were reduced in number and there were some vacuolated (endothelial) cells Intense fatty degeneration of the glomerular vessels and afferent arterioles was seen in frozen sections The epithelial wall of Bowman's capsule appeared normal but there were desquamated epithelial cells in the capsular space The tubules were irregularly collapsed atrophied and sometimes obliterated or dilated and filled with colloid cellular detritus desquamated epithelial cells and leucocytes Casts entirely formed of *polymorphonuclear* cells were abundant The tubular epithelial cells showed some fatty degeneration The arterioles and arteries were not thickened but there was a diffuse increase in the interstitial connective tissue especially in the cortex and infiltration with lymphocytes and polymorphs

Giglioli's cases IV and V belonged to the other group Case IV a girl of nine years of age had a malarial history of five years infection with frequent recurrences of *P malariae* infection and a successfully treated episode of oedema and proteinuria 18 months before admission the presenting oedema developed 12 days before admission there were no parasites in the blood Case V was a man 39 years of age who had a malarial history (*P malariae*) of two years and a history of intermittent slight oedema for the 10 months previous to admission *P malariae* was present in the blood The kidney lesions were much the same in both cases The kidneys were not enlarged They were scarred and the capsules were adherent The cortex was reduced in thickness Glomerular changes were prominent The tufts were matted and indistinct and adhesions to Bowman's capsule common Fatty degeneration of the endothelial cells was pronounced Some glomeruli were completely replaced by fibrous tissue The interstitial fibrous tissue was irregularly and diffusely proliferated and irregularly infiltrated with lymphocytes Polymorphs were rare There were foci of round cells about some of the vessels the walls of which were hypertrophied Many of the tubules were atrophied or destroyed In some areas the tubules were dilated and lined with flattened epithelium The lumina were filled with colloid and desquamated fatty cells Hyaline casts were numerous

Giglioli considered that his cases showed more serious involvement

of the glomeruli than of the tubules the lesions of which he believed to be largely secondary to the changes in the interstitial connective tissue and glomeruli On this basis he regarded them as examples of chronic glomerular nephritis in the active and terminal stages of development In his view the evidence indicated that in the early stages of this chronic disease in *P malariae* infections the renal lesions were primarily degenerative whereas in the later stages they were principally inflammatory and proliferative

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to the medulla the cortex was frequently anaemic and in most organs there was some degree of congestion sometimes intense and often irregularly distributed of the medullary vessels (Maegraith and Findlay 1944 Maegraith 1944)

Histological changes

(a) Glomeruli

Most authors have reported normal or only slightly changed glomeruli in blackwater fever whether the patient had died in an anuric state or not Some workers have described general slight congestion becoming intense in a few glomeruli (Dudgeon 1920) but more commonly they have commented on the relative bloodlessness of the tufts even when occasional glomeruli have been congested Paterni (1928) for instance found in the same specimen some glomeruli intensely engorged and others completely devoid of blood Haemorrhages into the glomeruli have been reported but these are uncommon An increase in cells has been recorded in a few glomeruli in kidneys in which they were otherwise apparently normal (Stephens and Christophers 1901) Gross changes in glomeruli have been noted only by Gouzien (1911) who claimed that a glomerular nephritis was present In the West African cases referred to above glomerular changes were few The tufts were occasionally congested but were more frequently anaemic and haemorrhages were not observed

Bowman s capsules are often apparently normal but they may be distended and occasionally such distension is a prominent feature of the histological picture (Thomson 1924 Whipple 1909) The epithelium sometimes shows mild or severe degenerative changes and may even appear necrosed Desquamation is not uncommon and may sometimes be considerable Occasionally the tufts may be adherent and young fibrous tissue has been described occupying the capsular space under these circumstances (Stephens and Christophers 1901) The capsular spaces are frequently empty but they may contain amorphous hyaline or granular material sometimes staining like haemoglobin sometimes giving the Prussian blue reaction for iron The capsules may occasionally contain considerable masses of material Thus Marchand (1918) records broad crescents of reddish brown partly granular partly hyaline material According to Dudgeon (1920) blood debris may be present in acute cases but blood cells have not apparently been recorded Sometimes the basement membrane has

been described as degenerate (Porak 1918 Ameuille *et al* 1918 Salvioli 19.. Rapaport 19.8 Hoeppli 19.9 Stephens 1937)



FIG 12—Glomerulus in a case of anur[illegible] bl[illegible]ckwat[illegible] [illegible]er Note the n[illegible]rmal cellul[illegible]ty the absence of erythrocytes in th[illegible] tuft [illegible] and the [illegible] or epitheli[illegible] changes Th[illegible] cap[illegible]ule [illegible] tended The dia[illegible] t [illegible] con[illegible]lut[illegible]d tubules sh[illegible]w [illegible]generative cha[illegible]ges [illegible] th[illegible] [illegible]pithel[illegible]m

(b) The tubules

1 Changes in the epithelium

(*a*) *Convoluted tubules* Changes in the epithelium of the convoluted tubules are nearly always present in blackwater fever whether the case has become anuric or not A few authors have observed no evidence of change in the epithelium (Porak 1918) and in cases which have progressed for some days hypertrophy and regeneration of the cells has been reported (Ameuille 1918) Most authors however have observed degenerative changes ranging from cloudy swelling and vacuolation (Dudgeon 19.0 Dudgeon and Clarke 1917) to complete necrosis of both cytoplasm and nucleus (Whipple 1909 Barratt and Yorke 1909 Paterni 19.8 Maegraith and Findlay 1944) The distribution of the degenerative changes is often patchy Degeneration and necrosis of the cells of both proximal and distal convoluted tubules has been frequently noted the more severe lesions being usually found in the latter The affected cells have been described as flattened (Barratt and Yorke 1909) or thinner than normal so that the tubular lumen appears dilated or with jagged edges projecting irregularly into the lumen The striation of luminal surface of the cells is lost early Degenerate cells are frequently desquamated and many authors have

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There appears to be little that is specific about the nature of the lesions which include all gradations of degeneration from cloudy swelling to granular degeneration and necrosis The granules in the degenerate cytoplasm sometimes stain pink with eosin and resemble haemoglobin droplets but there is no firm evidence of their being haemoglobin or derived from it They may or may not give the Prussian blue reaction for iron Malarial pigment is rarely if ever present

2 Changes in the tubules

The tubules have often been described as dilated sometimes grossly (Whipple 1909 Werner 1907 Barratt and Yorke 1909 Salvioli 1922 Thomson 1924) but although this is the appearance given by histological specimens actual measurements do not seem to have been made so that some of the so-called dilatation may in fact be the result of the frequent shedding or partial desquamation of the epithelial cells into the lumen In the tubules of the medulla the dilatation may be very obvious especially in regions of capillary congestion where there have been haemorrhages directly into the tubules which become filled with blood cells

The lumen is frequently filled with material usually described as casts varying in appearance from obvious desquamated epithelium and red blood cells to reddish-brown granules and spherules (sometimes but not always giving a positive iron reaction) the composition of which is uncertain The lumina are frequently described as plugged with such material and the assumption has in the past been too readily made that sufficient mechanical obstruction of this sort arises to account for the failure of urinary secretion (Foy *et al* 1943 Maegraith 1944) This point will be discussed later The material within the lumen is most abundant in the distal convoluted tubules the ascending loops of Henle and the collecting tubules (Werner 1907 Salvioli 1922 Maegraith and Findlay 1944) Its distribution thus differs from that of the epithelial cellular degeneration which is clearly marked in cells of the proximal as well as those of the distal convoluted tubule and less obvious in the collecting tubules

The contents of the lumina also change from the proximal to the distal extremities of the nephron In the proximal and distal convoluted tubules the contained material is usually more obviously cellular in nature consisting of desquamated cells and portions *of cells in various* stages of degeneration together with amorphous granular material which may be yellowish and not stain with eosin or, more commonly,

noted exposed basement membrane often itself involved in the degenerative process

The degenerate cells are usually described as granular the granules in the haematoxylin and eosin stained specimen being grey black rusty coloured reddish pink or yellowish They have often been said to be haemoglobin or its derivatives but the evidence for this is poor and such conclusions are based mainly on the similarity of the staining reactions of the granules and erythrocytes to eosin or alum haematoxylin The granules sometimes give a positive Prussian blue reaction for iron but not always even when they stain pink. The iron reaction is sometimes diffusely positive throughout the cytoplasm of the cells and may be positive in granules which fail to stain with eosin Hyaline colloid and diffuse fatty changes in the epithelial cells have also been reported Some authors describe large globular or irregular masses usually hyaline or colloid lying in the cytoplasm and distinct from the granules or the concomitant degeneration These masses for the same reasons as the granules have sometimes been described as containing haemoglobin Their true nature has not been determined In severe degeneration the nuclei undergo all the changes associated with necrosis (de Haan 1905 Werner 1907 Marchiafava and Bignami 1900 Barratt and Yorke 1909 Gouzien 1911 Ameuille *et al* 1918 Salvioli 1922 Paterni 1923 1928 Thomson 1924 Stephens 1937)

(*b*) *Cells of Henle s loops* The cells of the limbs of the loops of Henle are frequently degenerated those of the ascending limb being more commonly and more severely affected The degenerative changes are sometimes considerably less severe than those present in the cells of the convoluted tubules Thus the cells of the proximal limb have been described as showing finely granular degeneration while those of the distal limb show coarse granular changes The Prussian blue reaction for iron gives irregular results

(*c*) *Cells of collecting tubules* The cells of the collecting tubules are not frequently seriously affected They may show signs of degeneration and may sometimes desquamate into the lumen but they are often described as intact The iron reaction is variable and pink eosin-staining granules may appear in the cytoplasm similar to the granules seen in the cells of convoluted tubules (Stephens 1937)

The degenerative and necrotic cellular changes in the nephron are thus most prominent in the convoluted tubules especially the distal convoluted tubule and in the distal loop of Henle The cells of the collecting tubules are damaged less in proportion

There appears to be little that is specific about the nature of the lesions which include all gradations of degeneration from cloudy swelling to granular degeneration and necrosis The granules in the degenerate cytoplasm sometimes stain pink with eosin and resemble haemoglobin droplets but there is no firm evidence of their being haemoglobin or derived from it They may or may not give the Prussian blue reaction for iron Malarial pigment is rarely if ever present

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eosinophilic In the ascending loops of Henle and particularly the collecting tubules the granules are larger and may reach the size of an



Fig 13—Anuric blackwater f ver Section through the re al medull showing tubul r ch ges and granular m teri l in the tubular lum ina

erythrocyte or become agglomerated into large irregularly shaped masses Erythrocytes have been described in all parts of the nephron but they are very rare in the convoluted tubules and commonest in the collecting tubules usually in areas in which there is capillary congestion (*Stephens 1937 Maegraith and Findlay 1944*)



Fig 14—Non-a uric bl ckwat r f ver S ction th o gh the renal med ll showing tub il ch nges nd g *nular materi l the tub l r lum na*

The cellular debris the granules spherules and clumps of reddish-yellow material and erythrocytes are the commonest substances found in the tubules It would be tedious to do more than summarize other findings Thus grey crumbling masses agglutinated or clotted red cells fragmented red cells dirty red granules not staining with eosin hyaline blocks coagulated serum or albuminous material have been described at various times Occasional polymorphs have also been recorded

The material in the tubules has usually been said to be stained with or even composed of haemoglobin or its derivatives As in the epithelial cells the evidence for the presence of haemoglobin is based mainly on the staining reactions of the material the haemoglobinous matter staining like red cells with haemotoxylin and eosin or dark with iron alum haemotoxylin (Barratt and Yorke 1909) and yellow with picric acid (Thin 1899) Casts in the loop of Henle have been stated to give the haemoglobin reaction (Salvioli 19--) and granules of methaemoglobin have been reported (Paterni 19-3)

(c) Changes in other tissues

The relatively anaemic cortex and glomerular tufts and the irregular medullary capillary congestion have already been referred to In some areas as has also been mentioned the medullary congestion may be severe with all the appearances of the dilated and engorged vessels having broken through into the neighbouring degenerate tubules causing haemorrhage into the lumina which are dilated with free erythrocytes Multiple tubular haemorrhages of this sort are common in some cases Haemorrhages also occur in the interstitial tissues They are commonest and largest in the immediately subcapsular region and may be confined to the cortex even when the latter is anaemic but may also be found scattered throughout the medullary substance usually in direct relation to areas of congestion and damaged tubules especially the collecting tubules In the cortex conical haemorrhagic areas have been described with the base beneath the capsule and the apex pointing towards the medulla Such areas may possibly be small infarcts but they are not associated with any evidence of vascular obstruction by parasitized or damaged red cells

The interstitial tissue is sometimes oedematous Whipple (1909) for instance observed oedema in five of twelve cases and in the West African cases referred to elsewhere oedematous separation of the cellular matrix was sometimes observed Infiltration of the tissue with cells has also been described particularly around the blood vessels including

the large veins usually those of the medulla Paterni (1923) has for instance described lymphocytic infiltration about the vessels and Marchiafava and Bignami (1900) referred to nodules of epithelioid and leucocytic cells among the tubules of the medulla Lymphocytic accumulations in relation to the medullary vessels were not uncommon in the recent West African cases Biggs (1945) has suggested that they may be allergic in origin

Evidence of phagocytosis has been occasionally described malaria pigment appearing in the endothelial cells of the glomerular tufts and occasionally in the epithelial cells of the tubules Marchand (1918) described in one case large monocytic cells filled with haemosiderin concentrated in the cortico-medullary zone

The general picture in blackwater fever

Some of the information outlined above is conflicting but most authors agree on certain details With few exceptions they have found few changes in the glomeruli compared to those in the tubules Occasionally congestion of the glomeruli and even haemorrhages into the tufts have been described but more frequently the glomeruli have been found normal unaltered or anaemic The cortex has sometimes been found congested but more often pale and relatively anaemic Most severe changes have been recorded in the uriniferous tubules the degenerative and necrotic lesions of the epithelium being most prominent in the convoluted tubules and the ascending loop of Henle The lumina of the tubules especially those of the distal convoluted tubules the ascending loop of Henle and the collecting tubules have been found irregularly filled or plugged with casts composed of epithelial debris desquamated cells granular and hyaline material and red blood cells Most authors have reported the contents of the lumina stained with or composed of haemoglobin or its derivatives The vessels of the medulla have been found irregularly congested and haemorrhages reported into the interstitial tissue especially about the tubules

CHAPTER VIII

THE KIDNEY IN MALARIA AND BLACKWATER FEVER

(ii) *Pathogenesis of renal lesions*

PATHOGENESIS IS BLACKWATER FEVER The r le f haemoglob n — Th th ory f mechanical b truct — Effe ts of cul u g hae oglob . PARASITES IN THE PATHO ENESIS OF MALARIA AND BLACKWATE FEVER To — H emo o n SENSITIVITY AND ANTIGEN-ANTIBODY RE ACTIO S RENAL FUNCTION IN RELATION TO C ES IN E ECTROLYTE WATER PLASMA BA ANCE B OOD PRESSURE AND BLOOD VOLUME CHANGES IN RELATION TO REN L FUNCTION THE RENAL CHANGES IN MA ARIA AND BLACKWATER FEVER AS EXAMPL S OF THE EFFECTS OF CO TICAL ISCH EMI AND REN L ANOXIA Th p thol l les ons Th d el pment of — R al blood flow in e al a xia — Ch ges n enal blood fl w RECAPITULATION CORTICAL ISCHAEMIA AND RENAL ANOXIA IN MALAR A ND B A KWATER FEVER

WITH the exception of the so-called chronic interstitial nephritis described by Giglioli (1932) the renal lesions of acute chronic recurrent and repeated malaria have many points in common with one another and with those of blackwater fever In all the cortex is commonly pale and the glomeruli little affected the degenerative and necrotic lesions are most severe in the tubules especially the convoluted tubules the lumina of the convoluted tubules and the less damaged collecting tubules contain desquamated cells cell debris grey yellow or pinkish granules hyaline material or erythrocytes In the case of blackwater fever the tubular contents are generally believed to be stained with or composed of haemoglobin and its derivatives Apart from this the lesions of blackwater fever are identical in type and distribution if not always in degree with those of acute malaria

The changes in the kidneys are predominantly degenerative and not inflammatory Occasionally some cellular infiltration (usually perivascular or nodular) of the interstitial tissue has been reported but extensive inflammatory changes in the sense of vigorous cellular reaction in the interstitial tissue and glomeruli have been recorded only by Giglioli in his series of five cases with histories of chronic quartan malaria As mentioned elsewhere Giglioli s findings may have been complicated to some extent by the effects of secondary infection and the age of his patients He believes that the renal lesions are essentially degenerative in their early stages and become prevalently inflammatory and proliferative only in the later stages Whatever the explanation of the lesions observed in Giglioli s cases they are

unique in descriptions of the kidney changes in malaria All other workers are agreed that the damage is mainly concentrated in the tubular epithelium and not in the glomeruli and that the lesions are primarily degenerative and not inflammatory For the purposes of classification such lesions fall into the group of renal changes characteristic of the toxaemic kidney or nephrosis (Langdon-Brown and Evans 1946)

Although the pathological pattern is in many ways constant in that the lesions *are degenerative and concentrated in the epithelium* of the tubules (particularly those lying in or near the cortex) the clinical syndromes observed are not uniform As we have seen the patient may suffer from no obvious renal damage except for proteinuria and the passage of casts he may gradually pass into a state of hydraemic nephrosis uncomplicated except terminally by anuria or raised urea nitrogen or he may develop acute renal failure associated with anuria and raised blood urea nitrogen but not accompanied as a rule by any appreciable degree of haematuria It is difficult at first to appreciate how hydraemic nephrosis and azotaemic syndromes can derive apparently *from the same pathological changes in the kidneys* The most reasonable explanation appears to be that the azotaemic state becomes grafted on to the nephrosis as the result of the failure of urinary secretion The suppression of urine arises from physiological depression of the glomerular blood flow and consequent cessation of filtration No specific pathological lesions result from this redistribution of blood flow but as a result of it the already existing cortical ischaemia is increased and the tubular damage heightened in proportion The reversible nature of this kidney failure distinguishes it from acute nephritis (Maegraith *et al* 1945)

The tubular lesions of the nephrosis can in themselves be most readily interpreted in terms of intrarenal redistribution of blood flow They occur most prominently in the cortical portions of the tubules particularly the proximal and distal convoluted tubules and are as has been shown above usually associated with a cortex which compared to the medulla is relatively anaemic They can be reproduced experimentally in animals by interference with the renal circulation The development in malaria and blackwater fever of some degree of cortical ischaemia is thus probably the basic phenomenon involved in their production In mild degree such ischaemia gives rise to the epithelial changes and the clinical picture of the hydraemic nephrosis but when the ischaemia develops to the extent of limiting blood flow through the glomerular tufts anuria and the azotaemic syndrome appears

The arguments in support of this vascular conception of the pathogenesis of the renal changes in malaria and blackwater fever have been most thoroughly pursued in the latter disease but they apply equally well to the former It is proposed here to introduce and develop them from the point of view of blackwater fever

Other factors of course probably play some part in the production of the tubular lesions and possibly also in the development of anuria It is well known for instance that tubular epithelial degeneration and necrosis can be produced in animals by the injection of certain chemical poisons A specific toxic substance derived from the parasitic infection in malaria has often therefore been postulated and has been searched for but never found In spite of this failure to identify malarial toxins it is a clinical fact that the degree of renal damage and type of kidney failure developing in an individual case depend upon the species of invading parasite Thus acute azotaemic syndromes appear occasionally in acute *P falciparum* malaria and commonly in blackwater fever but are very rare in *P malariae* or *P vivax* infections The hydraemic nephrosis syndrome is much commoner in *P malariae* than in *P vivax* infections and is exceptional in *P falciparum* infections Most authors consider that the differences between the syndromes produced by the various infecting parasites are fundamentally related to the severity of the infection and the tendency or otherwise to latency and relapse In the acute and severe clinical states of malignant tertian malaria and blackwater fever for instance the conditions leading to anuria more easily develop than in the long continued relatively milder benign tertian and quartan attacks In the latter however the milder degree of injury to the kidney persists for long periods and gradually gives rise to irreversible kidney changes Malarial pigment has been implicated but not convincingly In anuric blackwater fever the urinary suppression has been incorrectly held to result mainly from mechanical blockage of the tubules with debris and precipitated haemoglobin products These theories are discussed below

THE PATHOGENESIS OF THE KIDNEY CHANGES IN BLACKWATER FEVER

The renal symptoms of blackwater fever are characterized by their acuteness and severity They differ from those of malaria mainly in the frequency of the appearance of anuria and in the passage in the urine of haemoglobin and associated pigments The anuria and haemoglobinuria are sufficiently dramatic phenomena to overshadow in the

minds of most observers the concomitant tubular failure Nevertheless evidence of tubular damage is found in most cases Proteinuria invariably accompanies the haemoglobinuria and is usually associated during the passage of haemoglobin with large numbers of hyaline and granular casts and quantities of cellular debris Proteinuria and cylindruria may appear before the passage of haemoglobin for example during the attack of malignant tertian malaria in which blackwater fever develops After haemoglobinuria has ceased the proteinuria may persist for some days the protein and casts slowly disappearing

The presence of casts indicates some degree of tubular damage and there is strong corroboratory biochemical evidence of this In the haemoglobinuric phase and for some time after haemoglobin has disappeared from the urine there is evidence of reduced urinary concentration The urine passed is of low specific gravity except during haemoglobinuria and heavy proteinuria it is usually low in concentrations of chloride (as sodium chloride) and urea particularly in the post-anuric period The sodium chloride concentration of the urine may be low before the onset of haemoglobinuria The low urinary chlorides are frequently associated with a fall in the blood chlorides but there is no direct relation between these concentrations The urinary chlorides are usually lowest during or immediately after the passage of haemoglobin but this is not always the case For instance Ross (1932) records one patient in whom the concentration of urinary chlorides was 0 15 per cent on the second and last day of haemoglobinuria and 0 44 per cent three days later and another patient in whom the chloride concentration was 0 09 per cent on the second and last day of haemoglobinuria and 0 05 and 0 06 per cent on the two succeeding days The urinary chloride concentrations in two cases immediately before the onset of oliguria were 0 42 and 0 50 per cent respectively A reduction of urinary urea nitrogen has also been recorded even in cases in which the blood urea has risen considerably This failure to concentrate urea is especially well seen during the polyuria which develops in cases recovering from anuria For instance in a case in the West African series during the recent war the urinary urea nitrogen was only 0 7 per cent five days after the restoration of urinary flow following a period of some days of almost complete anuria (Maegraith 1944) Wakeman (1929) records a similar case in West Africa in which during the first week after urinary flow was established the urinary urea concentration did not exceed 0 4 per cent although the blood urea nitrogen was over six times normal He considered this most striking evidence of injury to the tubular cells

The urea concentration test (Owen and Murgatroyd 1928) in one case showed impaired renal function Similar indications of dysfunction were obtained by Georgopoulos (1933) in water concentration tests carried out in a case six days after recovery of urinary flow following a short period of haemoglobinuria and anuria (Krauss 1904 Barratt and Yorke 1909 Ross Thomson and Simpson 1910 Gouzien 1911 Paterni 1923 Lahille 1915 Weselko 1926 Owen and Murgatroyd 1928 Yorke Murgatroyd and Owen 1930 Wakeman 1929 Wakeman and Morrell 1929 Ross 1932 Georgopoulos 1933 Foy Altmann Barnes and Kondi 1943 Macgraith 1944)

It is thus clear that the renal syndrome of blackwater fever has three component parts i e haemoglobinuria and proteinuria oliguria and anuria and tubular dysfunction indicated by cylindruria and reduction in urinary concentration As has been explained above there is evidence to show that the tubular damage is similar to that seen in malaria In order to establish the close relation between the malarial and blackwater fever renal lesions however it must first be shown that the latter are not specific in their origin and do not develop primarily from the haemoglobinuria or from the oliguria and anuria

The role of haemoglobin

The development of tubular dysfunction is obviously not dependent on the appearance of urinary suppression since signs of tubular injury appear in all cases of blackwater fever whether or not they become oliguric or anuric and in anuric cases frequently appear before the onset of suppression The fact that tubular dysfunction may precede and follow haemoglobinuria is strong presumptive evidence of its non-specific origin

The theory of mechanical obstruction

Until recently it was generally accepted that the renal lesions arose as a result of the passage of the blood pigments through the kidneys Barratt and Yorke (1909) and Yorke and Nauss (1911) produced haemoglobinuria in rabbits by intravenous injection of haemoglobin solutions and found that some animals developed anuria The kidneys of these rabbits showed changes similar to those seen in blackwater fever the prominent feature being tubular epithelial damage and the accumulation of granular pink material in the lumina of the tubules They concluded that the anuria had probably arisen from mechanical obstruction by plugs of this material (which they considered was

derived from haemoglobin) to the flow of urine along the tubules which were frequently widely dilated as if from pressure Although they held that this mechanical obstruction was the main factor at work they pointed out that similar failure of urinary flow might arise from any agency which caused a diminution of glomerular filtration such as lowering of hydrostatic blood pressure or dehydration If the blood volume were maintained in the experimental animals they found that relatively large quantities of haemoglobin could be injected without initiating anuria Lesions were readily produced in dehydrated animals fed on a dry diet They considered that the precipitation of the haemoglobinous material in the tubules was closely related to the degree of concentration of the urine

Baker and Dodds (1925) followed up the work of Yorke and Nauss by investigating the factors controlling the precipitation from solution of haemoglobin and its derivatives *in vitro* and after injection into rabbits They found that a solution of rabbit blood haemoglobin obtained by alternate freezing and thawing of erythrocytes was lethal on injection unless adequately filtered Filtered solutions of haemoglobin (obtained by laking rabbit erythrocytes with asbestos fibre) were injected into rabbits at repeated intervals and it was found that the urine passed was brown and cloudy with precipitate when acid and bright red and clear when alkaline Animals passing brown urine suffered kidney damage those passing red urine did not They found it was impossible to produce renal obstruction by injecting haemoglobin into rabbits unless the animals are kept short of green food On greens the urine was strongly alkaline on the dry diet used by Yorke and Nauss the urine was acid Baker and Dodds concluded that the urinary pH was the chief factor deciding the precipitation of haemoglobin and its derivatives from solution in urine They considered pH more important than the volume or concentration of the urine the importance of which had been emphasized by Yorke and Nauss

Experiments with solutions of haemoglobin *in vitro* confirmed their view that precipitation was facilitated by a low pH They also observed that provided the urine was sufficiently acid the precipitation of the haemoglobin was dependent on the presence of sodium chloride the optimal concentration of this electrolyte being about 1 per cent The precipitate formed in saline and acid solutions of haemoglobin contained haematin and they therefore suggested that in the animal a similar precipitation took place in the tubules acid haematin being deposited when the urinary pH and saline concentration became

suitable They argued that this precipitation led to mechanical obstruction to urinary flow and consequent impairment of renal function They considered that their experiments showed that the affected animals could get rid of plugs in the tubules and that similar flushing of debris accounted for the failure of blocking to occur in such conditions as paroxysmal haemoglobinuria and those cases of blackwater fever which did not become anuric or which recovered from anuria They believed that the severity of the renal lesion was thus dependent in part on the degree of haemoglobinuria As will be seen these views are not in accordance with clinical experience of blackwater fever

Baker and Dodds concluded from their experiments that since the factors leading to precipitation of pigment are the acidity and salt concentration of the urine any type of therapy tending to reduce these should prove of value This statement has been one of the most influential made in the history of blackwater fever for upon it has been built up the concentrated alkali therapy which has been adopted as a standard method of treatment and prophylaxis against renal failure in blackwater fever for the last 20 years In that period there has been no substantial change in the death rate for the disease (Maegraith 1944) and from time to time the basis of the alkali therapy has been challenged but not before it has become widely employed in the treatment of other examples of the renal anoxia syndrome

Ross (1932) conducted further experiments on the precipitation of haemoglobin from solution at various pH levels and salt concentrations and from his results and his clinical experience of blackwater fever concluded that the importance of sodium chloride as the main electrolyte capable of precipitating haemoglobin derivatives from an acid urine had been exaggerated In his cases as has been already pointed out the sodium chloride concentration of the urine was usually low and always considerably less than the optimal concentration referred to by Baker and Dodds Ross could not agree that the appearance of suppression was dependent principally either on the salt concentration or the pH of the urine and inclined to the view that Yorke and Nauss had held namely that the degree of concentration of the urine was of primary importance

Georgopoulos (1933) surveyed the literature and his own experience and concluded that the mechanical factor could not be considered the sole cause of oliguria or anuria in blackwater fever In many cases the degree of blocking of the tubules was not enough to account for anuria Georgopoulos pointed out the rarity of anuria in other

conditions associated with haemoglobinuria and stressed the greater significance of the impairment of renal function indicated by the passage of dilute unconcentrated urine in blackwater fever

Foy *et al* (1943) made a very careful analysis of the information available and showed that the theory of mechanical blockage was untenable They considered that the production of anuria could not be accounted for by any single factor but was the result of several including dehydration diminished blood volume and diminished renal circulation and glomerular filtration

Maegraith (1944) Maegraith and Findlay (1944) and Maegraith and Havard (1944) came to much the same conclusion as Foy *et al* with regard to mechanical obstruction They pointed out that if the suppression of urinary flow arose from mechanical blocking of the tubules arising from the precipitation of haemoglobin derivatives the urine according to Baker and Dodds must be acid must contain haemoglobin and must have a concentration of sodium chloride of about 1 per cent Further the number of blocked tubules must be very large approaching 100 per cent Anuria should occur more frequently after a severe haemolysis than after a light one provided the urinary conditions were suitable Moreover after recovery of urinary flow following anuria the urine should be loaded with flushed out plugs of pigment and casts and should be normal in composition

A survey of the literature and their West African cases showed many facts which were inconsistent with the blockage theory The urine at the onset of anuria is as often alkaline or neutral as it is acid Anuria in some cases develops after the haemoglobinuria has ceased or may fail to develop in the presence of heavy and persistent haemoglobinuria It has already been pointed out that the sodium chloride concentration in the urine is low and never as great as 1 per cent The degree of blockage of the tubules has not been ascertained accurately by any worker but it is often clearly insufficient to account for complete failure of urinary flow In some cases with very considerable blockage the urinary flow remains unaffected Finally in the post-anuric phase of the disease the first urine passed subsequent to anuria is usually but not always (Wakeman 19-9) clear and free from the flushings of debris and casts which would be expected

Maegraith and his colleagues have also stressed other inconsistencies For instance the presence and distribution of the tubular epithelial damage and the associated urinary dilution are difficult to explain on the basis of mechanical obstruction

Journe (1944) considered that the cause of anuria in blackwater fever

was not yet determined He believed that precipitation of haemoglobin or its products might play a part but was not the whole explanation His arguments were similar to those of the workers referred to above but he also pointed out that it is very difficult to explain the abrupt onset of anuria in terms of tubular obstruction unless a sudden and simultaneous blocking occurs which he regarded as extremely unlikely He emphasized the importance of the loss of concentrating power of the kidneys in the post-anuric phase and suggested that recovery of urinary flow was the outcome of a sudden restoration of glomerular filtration and was followed by slower recovery of the damaged tubules

Peters (1945) and others have also noted the inadequacy of the theory of mechanical obstruction and it is now becoming generally accepted that although some obstruction to urinary flow may arise in certain nephrons as a result of the accumulation of material in the lumina this obstruction is of minor importance and is not the chief factor concerned in the initiation or maintenance of oliguria or anuria

The effects of circulating haemoglobin

In searching for a more satisfactory explanation than mechanical obstruction to account for the kidney changes in blackwater fever workers have paid particular attention to the effects of circulating haemoglobin on renal function

There is little evidence that the passage of small quantities of haemoglobin damages the glomerular membrane Foy *et al* (1943) have carefully reviewed the literature on this point According to Monke and Yuile (1940) about 3 per cent of the pores of the normal glomerular membrane are large enough to permit the passage of the haemoglobin molecule which under certain circumstances may dissociate into smaller molecules which are more easily filtered (Steinhardt 1938) When haemoglobin is present in the plasma therefore it can be passed through the glomerulus without serious injury to the membrane Several authors have found that within certain limits haemoglobin acts as a threshold substance in man and some other animals appearing in the urine only when the plasma concentration has reached a threshold value of roughly 70 mgm per cent a figure which varies considerably in individual subjects and according to individual workers (Barratt and Yorke 1909 Lichty *et al* 1932 O Shaughnessy *et al* 1939 Ottenburg and Fox 1938 Fairley 1940 Gilligan and Blumgart 1941) Lichty *et al* produced experimental evidence in dogs indicating that some haemoglobin was reabsorbed by the epithelial cells of the con-

voluted tubules They held that haemoglobinuria did not appear until the reabsorptive capacity of the epithelium was exhausted a view which has received some support from other workers (Gilligan and Blumgart *1941* Yuile *et al* *1941*)

In view of the findings discussed above Foy *et al* pointed out that functional or structural damage to the glomerular membrane need not be postulated in order to account for the passage of haemoglobin in the urine Nevertheless some change in permeability must take place since haemoglobinuria is always accompanied by heavy proteinuria and the passage of protein in large quantities is not a physiological occurrence No doubt some of the protein passed may be accounted for by the effects of fever and other equally non-specific features of the blackwater fever attack but the maximum amounts are passed contemporaneously with the haemoglobin and the quantity discharged is reduced rapidly after the cessation of haemoglobinuria It is possible therefore that circulating haemoglobin may affect the membrane sufficiently to alter its permeability to protein such as albumin of approximately the same molecular weight Hesse and Filatov (1933) obtained evidence suggestive of this in dogs injected with homologous haemoglobin in which albuminuria was found to precede the appearance of haemoglobinuria

The physiological effects of the injection of solutions of haemoglobin and allied pigments have been investigated by numerous workers in man and animals The results have been found to depend largely upon the animal used and the conditions of the experiments Some of this work e g that of Baker and Dodds has already been referred to above There is in addition a considerable amount of other experimental evidence which has a bearing on the pathogenesis of the renal lesions in blackwater fever although it is primarily concerned with other conditions in which the renal lesions are essentially the same (Bywaters and Beall 1941 Bywaters and Dible 1942 Maegraith 1944 Maegraith Havard and Parsons 1945) particularly the anuria of incompatible blood transfusion (in which haemoglobin is circulating) and that of crush injury (in which the pigment concerned is myoglobin)

Many workers have found that under some conditions the injection of large quantities of homologous or heterologous pigment (haemoglobin or myoglobin) with or without the cell debris removed may produce no toxic effects (Bayliss 19-0 Baker and Dodds 19-5 Borchardt and *Tropp 1928 Ottenburg and Fox 1938 O Shaughnessy* 1939 Fairley 1940) Clinically it has also been shown in blackwater

fever incompatible transfusion and various forms of haemoglobinuria that large quantities of haemoglobin may pass through the kidney without inflicting serious damage to the renal tissue and without apparent interference with kidney function (Maegraith *et al* 1945 Yuile 1942)

On the other hand at times the passage of haemoglobin or myoglobin does appear to lead to renal dysfunction and parenchymal changes Yorke and Nauss (1911) succeeded in producing renal lesions in rabbits following injections of homologous haemoglobin solutions but the lesions developed only if the animals were kept on a dry diet Rabbits on an ordinary green vegetable diet excreted haemoglobin rapidly and withstood injection of large quantities without ill effect As described above Baker and Dodds (1925) confirmed these experimental results and considered that to obtain renal lesions in rabbits it was necessary to keep the urinary reaction acid

De Gowin (1934 1938) and his colleagues obtained similar results in dogs injected with solutions of haemoglobin Animals given ammonium chloride and consequently passing an acid urine developed severe tubular lesions and two died from anuria and renal failure although five showed no decrease in urinary flow Animals passing alkaline urine showed neither renal failure nor tubular epithelial changes De Gowin considered that the acidity of the urine was an important element in the development of the renal damage but as Foy *et al* (1943) point out other factors the significance of which de Gowin did not stress such as dehydration and the effect of the ammonium chloride on the blood base were also involved in his experiments and make the interpretation of them difficult

De Navasquez (1940) repeated the animal experiments of Baker and Dodds and found that rabbits injected with haemoglobin solution showed no evidence of blockage of the renal tubules even when passing acid urine He considered dehydration was probably more important than the acidity of the urine in the pathogenesis of renal failure associated with the urinary passage of haemoglobin

Corcoran and Page (1945) watched the effects of the injection of myoglobin haemoglobin and haematin in conscious trained dogs taking a diet giving rise to an acid urine They followed the total renal and glomerular blood flow and found that intravenous injection of myoglobin or haemoglobin brought about renal injury in aciduric dogs They concluded that these pigments might therefore be significant factors in the pathogenesis of the renal changes in crush injury and incompatible transfusion This conclusion was qualified however

since in certain (then unpublished) experiments they were unable to obtain severe renal damage in hydrated aciduric rats following injection of myoglobin whereas similar doses of the pigment produced renal injury in dehydrated aciduric animals with crushed limbs They considered that the renal damage in their dogs resulted from tubular obstruction by myoglobin or its products and impaired tubular secretory activity arising from ingestion of the pigment by the epithelial cells and complicated by the histotoxic effects of the haematin liberated in the tubules As will be seen later their results can be interpreted differently and on the whole do not bear out the authors contentions

Bywaters and Stead (1944) investigated the toxicity of solutions of myoglobin in rabbits They injected pigment extracted from dog muscles into animals secreting an acid urine and injured by a standardized muscular compression Severe renal dysfunction was evidenced in only four of 25 animals injected Myoglobin injections had no effect on normal animals

In the experimental work described above it will be noted that successful results so far as the production of renal insufficiency and damage is concerned have depended on the existence of some state which is unnatural to the animal concerned In rabbits for instance renal lesions developed only in those animals passing acid urine and in those in which the urine was concentrated as a result of a limited intake of fluid in the diet Solutions of haemoglobin or myoglobin did not *per se* show signs of toxicity in normal animals Yuile Gold and Hinds (1945) have recently re-examined the problem on a wide experimental basis and have obtained results which are worthy *of close attention These authors reviewed the literature* and showed that syndromes of renal failure associated with intravascular haemolysis have several points in common In all there is a circulating pigment a factor of vascular or chemical nature influencing the organism as a whole and a renal lesion comprising epithelial degeneration and pigmented casts and debris in the tubules They injected solutions of haemoglobin (obtained from citrated rabbit blood) into rabbits secreting acid or alkaline urine (the reaction adjusted by diets essentially the same as those employed by Yorke and Nauss) and in which the renal tubules had been previously damaged by sodium tartrate or temporary clamping of the renal artery (Scarff and Keele 1943) Water was allowed freely to all animals

In normal animals injection of haemoglobin solution was not followed by signs of renal disturbance whether the urine had been

rendered acid or allowed to remain alkaline Brief clamping of the renal artery gave rise to haemoglobin casts in all animals rather more numerous in those with acid urine In two animals passing acid urine there was a rise of blood urea nitrogen shortly after the injection of haemoglobin one animal developed oliguria When the renal artery was clamped for a longer period the animals suffered more severe renal syndromes Two animals with acid urine became anuric and died There were haemoglobin casts in the renal tubules in these animals A third aciduric rabbit showed a rise in blood urea nitrogen without accompanying oliguria This animal was killed and found to have many tubular haemoglobin casts In the animals given tartrate maximal renal injury was observed in the aciduric animals

The authors concluded that the precipitation of haemoglobin in the tubules was not primarily dependent on the urinary reaction but upon a functional abnormality of individual nephrons indicated anatomically by the degenerative lesions of the tubules The latter appeared to be non specific since they could be produced by either ischaemia or chemical poisons (tartrate) However if the combination of tubular damage and haemoglobinuria existed they considered that the pH of the urine might be important since in their experiments pigmented casts appeared more commonly when the urine was acid

The experimental findings quoted above demonstrate very clearly the independence of the production of the tubular lesions and the development of anuria For instance only two of de Gowin s aciduric dogs developed anuria before death in the others the urinary flow was not greatly reduced In spite of this epithelial necrosis and blocking of the tubular lumina were prominent in all animals Other workers (for instance Yuile *et al*) obtained similar results Given the correct con ditions such as aciduria tubular lesions could be produced by adequate injections of haemoglobin but anuria did not by any means always accompany even the severest damage This distinction between the development of clinical evidence of tubular lesions and the appear- ance of anuria has been pointed out above in regard to blackwater fever (Maegraith 1944) and has been noted in similar clinical condi- tions such as incompatible transfusion and crush injury (Bordley 1931 Kimmelstiel 1938)

Foy *et al* (1943) have indicated the possible fallacies involved in arguing directly from experimental results in animals to humans and stressed the variations in the metabolism of haemoglobin which are found in various animal species (Foy and Kondi 1935 1938 Fairley 1941) Nevertheless the general pattern of the results of haemoglobin

injection in the animals so far studied is fairly uniform and conforms in the main with the picture drawn by Yuile *et al* It seems clear that under certain circumstances the intravenous injection of haemoglobin is followed by the development of tubular lesions and sometimes kidney failure Injection of haemoglobin into normal animals living on a normal diet has usually no toxic effects Other factors (for instance the secretion of acid urine or conditions of reduced fluid intake and decreased urinary output) must apparently be present before renal damage appears Such factors are not always present in blackwater fever and for this reason as well as for others already discussed it is extremely unlikely that the renal changes in this disease are initiated purely by the presence of circulating haemoglobin or its passage through the kidneys There is a strong probability however that haemoglobin may exaggerate the severity of the renal lesions under appropriate conditions particularly in view of its constricting effect on the kidney vessels and consequent ability to reduce the renal blood flow (Mason and Mann 1931 Reid 1929 Hesse and Filatov 1933)

The close anatomical similarity between the kidney lesions of blackwater fever and those of acute and chronic malaria has been noted elsewhere It is clear from the evidence just considered that the former do not owe their origin specifically to the passage of haemoglobin. Their similarity to the latter thus appears all the closer The non specific genesis of both is further suggested by their obvious likeness to the lesions seen in many other conditions of renal failure occurring in acute disease such as those recently grouped within what has been called the syndrome of renal anoxia (Maegraith *et al* 1945) In the discussion which follows the development of the renal changes in blackwater fever and malaria are therefore considered together

PARASITES IN THE PATHOGENESIS OF THE KIDNEY LESIONS OF MALARIA AND BLACKWATER FEVER

It has already been noted that in malaria parasites are usually scanty in the renal tissue although they may be numerous in the peripheral blood and elsewhere Only very rarely does the kidney contain parasites in appreciable numbers and stasis or thrombosis arising from vascular obstruction by parasitized erythrocytes is very unusual The parasites which are present are most plentiful in the intertubular capillaries of the medulla and are not found specifically in relation to the damaged tubules in the cortex Malarial pigment is also scanty and most con-

centrated in the glomeruli Both parasites and malarial pigment are rare in the kidneys in blackwater fever

It is clear therefore that parasites can have little or no direct influence on the development of the renal lesions in either malaria or blackwater fever The absence of severe vascular changes such as those seen in the brain is probably related as in the case of the liver to naturally high permeability of the endothelial walls No satisfactory explanation of the relatively low numbers of parasites found in the kidney has yet been provided

Indirect effects of the parasitic invasion are more difficult to determine There is little doubt however that the general anoxaemia arising from the destruction of red cells and the loss of active haemoglobin and oxygen from the oxyhaemoglobin of the invaded cells is an important factor in producing local tissue anoxia and plays a part in aggravating the degenerative changes in the tubules Other factors may also be involved two of which must be considered here namely (i) possible diffusable toxins derived from the parasites or their metabolic products and (ii) the products of schizogony especially haemozoin

(i) Toxins

The tubular epithelial degeneration has often been said to be the result of the action of malarial toxins of one sort or another including specific nephrotoxic substances liberated at the time of lysis of erythrocytes In the kidney where dense agglomerations of parasites are very much the exception rather than the rule local accumulation of the products of altered parasitic metabolism e g pyruvic or lactic acids cannot be of much importance Neither these substances nor parasitic toxins have been identified in the renal tissues Lintwareff (1937) suggested that the parasites might produce a toxin which could combine reversibly with haemoglobin to form a compound which acted directly on the tubular epithelium producing fatty and finally degenerative changes He attempted to establish his hypothesis by comparing the effects on the renal function of guinea pigs of the injection of solutions of haemoglobin obtained from normal and untreated malarial individuals Animals injected with malarial haemoglobin developed proteinuria those injected with normal haemoglobin did not Proteinuria was most intense in animals injected with haemoglobin from cases of *P falciparum* infection His results were not very convincing

injection in the animals so far studied is fairly uniform and conforms in the main with the picture drawn by Yuile *et al* It seems clear that under certain circumstances the intravenous injection of haemoglobin *is followed by the development of tubular lesions and sometimes* kidney failure Injection of haemoglobin into normal animals living on a normal diet has usually no toxic effects Other factors (for instance the secretion of acid urine or conditions of reduced fluid intake and decreased urinary output) must apparently be present before renal damage appears Such factors are not always present in blackwater fever and for this reason as well as for others already discussed it is extremely unlikely that the renal changes in this disease are initiated purely by the presence of circulating haemoglobin or its passage through the kidneys There is a strong probability however that haemoglobin may exaggerate the severity of the renal lesions under appropriate conditions particularly in view of its constricting effect on the kidney vessels and consequent ability to reduce the renal blood flow (Mason and Mann 1931 Reid 1929 Hesse and Filatov 1933)

The close anatomical similarity between the kidney lesions of blackwater fever and those of acute and chronic malaria has been noted elsewhere It is clear from the evidence just considered that the former do not owe their origin specifically to the passage of haemoglobin Their similarity to the latter thus appears all the closer The non-*specific genesis of both is further suggested by their obvious* likeness to the lesions seen in many other conditions of renal failure occurring in acute disease such as those recently grouped within what has been called the syndrome of renal anoxia (Maegraith *et al* 1945) In the discussion which follows the development of the renal changes in blackwater fever and malaria are therefore considered together

PARASITES IN THE PATHOGENESIS OF THE KIDNEY LESIONS OF MALARIA AND BLACKWATER FEVER

It has already been noted that in malaria parasites are usually scanty in the renal tissue although they may be numerous in the peripheral blood and elsewhere Only very rarely does the kidney contain parasites in appreciable numbers and stasis or thrombosis arising from vascular obstruction by parasitized erythrocytes is very unusual The parasites which are present are most plentiful in the intertubular capillaries of the medulla and are not found specifically in relation to the damaged tubules in the cortex Malarial pigment is also scanty and most con-

SENSITIVITY AND ANTIGEN-ANTIBODY REACTIONS

Renal symptoms occasionally develop in protein and anaphylactic shock (Longscope and Rackemann 1917) and on the analogy of Volhard's (1931) theory of the allergic nature of acute nephritis some authors have suggested that a sensitivity mechanism may be involved in the genesis of the renal deficiency syndromes of malaria and blackwater fever There is not much evidence in support of this hypothesis but it cannot altogether be disregarded especially in view of the periodic discharge into the plasma of protein and other foreign material at the time of parasitic sporulation

Haemoglobin does not appear to be involved in any sensitivity reactions since its effects are demonstrable after a single injection (de Gowin Warner and Randall 1938)

Allergic renal reactions usually involve the whole parenchyma and are concentrated mainly on the glomerular apparatus (Masugi 1933 1934) Specific damage to the epithelium such as is found in malaria is unlikely in such reactions It is possible however that some mechanism is involved similar to that suggested by Gear (1946) for the formation of autolysins The renal epithelium may be affected by the prevailing anoxia or directly as a result of the malarial attack and become capable of acting as an auto-kidney-epithelium antigen producing a diffusable anti-kidney-epithelium antibody Any resulting antigen-antibody reaction might give rise to degeneration and necrosis similar to that described by Schwentker and Comploier (1939) in rabbits after injection of mixtures of homologous kidney tissue plus coccal extracts No experimental evidence of such reactions is available in malaria or blackwater fever

RENAL FUNCTION IN RELATION TO CHANGES IN ELECTROLYTE-WATER PLASMA BALANCE

Although they cannot be regarded as initiating factors in the renal failure the changes in electrolyte-water balance occurring in the blood in malaria and blackwater fever probably influence the kidney function considerably particularly with regard to urinary output This is especially true in cases in which some degree of anhydraemia is developed as a result of loss of fluid from vomiting diarrhoea and sweating in circumstances in which the fluid intake is low Such cases are uncommon since in modern treatment fluid is usually given in adequate and sometimes excessive quantities (Paramore 1945

and other experiments aimed at identifying toxins have been equally unsuccessful

(ii) Haemozoin

Some authors have held that malarial pigment (haematin) is concerned in the production of the renal lesions and have provided experimental evidence in support of this contention Brown (1913) however came to the conclusion that it was not possible to correlate the renal complications of artificial haematin intoxication in rabbits with the changes found in the kidney in human malaria since the lesions produced by haematin showed a predominance of glomerular damage whereas those of malaria were essentially tubular He decided that the damage caused by the haematin resulted primarily from its effects on the small blood vessels which were dilated and congested and the endothelium of which was damaged

Anderson Morrison and Williams (1942) and Anderson and Morrison (1942) investigated the pathological changes following injection of ferrihaemate (haematin) into dogs and monkeys In both species they obtained renal lesions including congestion and thrombosis of the glomerular vessels and degeneration of the epithelium of the convoluted tubules The changes were less pronounced in monkeys in which they could be directly compared with malarial lesions All five monkeys injected with disodium ferrihaemate showed *histological evidence of renal damage The glomeruli* were not greatly affected the changes being most pronounced in the convoluted tubules and occasionally in the cells of Henle s loop and the collecting tubules The renal changes in monkeys which died from *P knowlesi* infections were much the same and similar to those described by Taliaferro and Mulligan (1937) The epithelial cells of the convoluted tubules always showed some degree of degeneration and there were accumulations of amorphous material in the tubular lumina and some glomerular spaces In some tubules there were hyaline and granular casts The authors concluded from the changes in the kidneys and other organs that although the parasitic pigment was capable of producing lesions in many respects similar to those of malaria it was unlikely that it was involved as a toxic factor in the disease since it was never liberated from the parasite in any quantity in a soluble form They concluded that the principal agent of injury in monkey malaria was probably anoxaemia resulting from vascular occlusion and severe anaemia

that the reduction in glomerular filtration resulted from anhydraemia which McCance and Widdowson (1937) pointed out might act by increasing the colloidal osmotic pressure of the plasma reducing the volume of circulating blood so that the usual number of glomeruli were not filled or by increasing the viscosity of the blood and so diminishing the glomerular blood flow It is clear however that anhydraemia cannot be the only factor concerned since Glass (1932) has obtained azotaemia and terminal renal failure in experimentally produced hypochloraemia in the absence of dehydration and a similar fall in glomerular filtration rate has been recorded in uncompensated alkalosis in which there was no anhydraemia or fall of blood pressure (McCance and Widdowson 1937 McCance 1936 Army Malaria Research Unit 1945) Wilkinson and McCance (1940) have recently further studied the creatinine and urea clearances in rabbits rendered salt deficient without anhydraemia (Michelsen 1933) and have shown that such deficiency results in azotaemia and oliguria on normal fluid intakes relative failure to excrete water on high fluid intakes and a reduction in glomerular filtration rate The latter in these experiments could be explained neither by the existence of anhydraemia nor reduction in hydrostatic blood pressure

Severe sodium chloride deficiency has thus been shown to bring about a reduction in glomerular filtration rate which is independent of the existence of anhydraemia McCance and Widdowson have suggested that this fall in glomerular filtration may develop from (i) a reduction in the number of functioning glomeruli arising from diminution in circulating blood volume or (ii) from a diminished renal circulation caused by increased blood viscosity or unknown factors Few workers would agree with the former suggestion since it is generally considered (although Winton (1937) believes the point is not settled) that variation of the numbers of active glomeruli such as is seen in frogs is not a characteristic of mammalian renal physiology (Richards and Schmidt 19-4 White 1939 Shannon 1939 Smith 1939-40 1937) The explanation of the changes in glomerular filtration is probably to be found in the general changes of cortical blood flow which will be discussed later

It was pointed out above that salt deficiency may be concerned in the development of the renal derangements in malaria and blackwater fever but it is probably only of minor importance and is not the initiating factor except very rarely since the reduction of this electrolyte is seldom within the limits believed necessary for the production of renal dysfunction (Glass 193-)

Bulletin of the U S Army Medical Department 1945) In anhydraemic cases including those in which the blood volume has been reduced by the appearance of medical shock the failure of urinary flow probably results from insufficiency of glomerular blood flow and filtration, but it is likely that the epithelial lesions are also aggravated to some extent by the diminished flow of urine through the tubules The significance of this diminished flow was first stressed by Yorke and Nauss who showed that saline injections could prevent anuria in aciduric haemoglobinuric rabbits fed on a dry oats and bread diet and has been repeatedly pointed out since by other workers Gersh (1936) for instance found that the kidneys of dehydrated rabbits injected with pig haemoglobin contained much larger amounts of tubular debris and haemoglobin than did those of rabbits not deprived of fluid. Foy *et al* (1943) suggest that the formation of tubular plugs may be facilitated in dehydration states since the concentration of urine is increased when the available water is limited and separation of precipitates therefore becomes more likely It sould be noted in this case that the tubular plugs form as a *result* of the diminished urinary flow (caused presumably by diminished glomerular filtration) and do not themselves initiate it

Plasma sodium chloride deficiency is probably a contributory factor in the production of renal failure in some cases of malaria and blackwater fever since it has been often shown experimentally and clinically to be capable *per se* of giving rise to derangements of kidney function Some authors consider that deficiency of sodium is more important than that of chloride and link the renal changes found in salt deficiency with dehydration arising from changes of electrolyte concentration in the tissue fluid which are independent of fluid intake (Kerpel-Fronius and Butler 1935 Gamble 19_9 1936) This cannot apply often to malaria or blackwater fever in which the deficiency frequently arises mainly from chloride loss following excessive vomiting

McCance and his colleagues in their careful studies of the effects of induced salt deficiency in man and other animals found in common with other workers that there was always an associated increase in blood urea nitrogen They suggested that this azotaemia resulted from a combination of a reduction in glomerular filtration rate increased re-absorption of urea (Blum 19_8 19_9) and a breakdown of body protein The reduction of glomerular filtration rate was shown experimentally by McCance and Widdowson (1937) and Harrison and Darrow (1939) and it was found by the former workers that there was no associated fall of blood pressure It was therefore suggested

of the pressure in the renal artery (Winton 1937 Fahr and Ershler 1941) so that the head of the general systemic pressure is not necessarily a measure of the glomerular pressure or of the glomerular filtration The latter appears much more to be a function of the blood flow through the tuft than of the hydrostatic pressure (Richards and Plant 192-) The urinary volume excreted may thus be regarded in part at any rate as a function of glomerular filtration and thus of glomerular flow so that failure of urinary excretion in malaria and blackwater fever (clinically manifested as oliguria or anuria) may in the absence of any evidence regarding increased re-absorption of fluid in the nephron be taken basically as evidence of reduction of glomerular flow The great importance of this will become clear in the argument outlined below

THE RENAL CHANGES IN MALARIA AND BLACKWATER FEVER AS EXAMPLES OF THE EFFECTS OF CORTICAL ISCHAEMIA AND RENAL ANOXIA

It has been shown above that the renal lesions of malaria and blackwater fever are essentially similar consisting primarily of degenerative changes in the epithelium of the tubules and the collection of debris and other material in the tubular lumina The epithelial degeneration is most pronounced in the portion of the nephron lying in or near the cortex i e the convoluted tubules and the ascending limb of Henle s loop Glomerular changes are seldom marked and the cortex is frequently anaemic in contrast to an irregularly congested medulla

The clinical signs of renal disturbance differ in malaria and blackwater fever since in the latter in addition to evidence of tubular involvement which is equally obvious in malaria the appearance of anuria (which is very uncommon in malaria) is frequent and is associated with azotaemia The signs of tubular derangement are believed to arise from the degeneration of the epithelial cells and the anuria from failure of glomerular filtration caused by reduction of the glomerular blood flow

It is clear therefore that any rational account of the genesis of the renal changes in the two diseases must cover the explanation of both the tubular degeneration and the failure of glomerular filtration As we shall see the only hypothesis that fulfils these conditions is one which postulates a redistribution of the intrarenal blood flow during the course of both malaria and blackwater fever whereby first relative cortical ischaemia and ultimately renal anoxia develop

Azotaemia and derangements of renal function associated with a reduction in glomerular filtration rate of inulin and creatinine have been reported in other states which may arise in malaria and blackwater fever and in which the plasma concentrations of electrolytes are disturbed Both acidosis and alkalosis for instance may affect renal function The former is uncommon in malaria but has been recorded in blackwater fever in the sense of a reduction of plasma alkali reserve (Fairley and Bromfield 1934) Some degree of alkalosis is probably common in cases of blackwater fever treated with modern excessive alkali therapy (Smith and Evans 1943) and no doubt exerts its damaging influence on the already potentially deranged kidneys of such cases (Foy *et al* 1943 Maegraith 1944 Maegraith and Havard 1944) The effects of such excess alkali have frequently been described in experimental and clinical work and recently in normal subjects given the high dosage of alkali recommended by some authors for the treatment of blackwater fever (Army Malaria Research Unit 1945)

BLOOD PRESSURE AND BLOOD VOLUME CHANGES IN RELATION TO RENAL FUNCTION

Gross reduction of hydrostatic blood pressure and general circulating blood volume such as occur in shock states and frequently gives rise to profound renal dysfunction associated with anuria are seldom seen *in malaria except in algid pernicious forms In blackwater fever the* hydrostatic blood pressure may fall considerably just before the onset of anuria but this is by no means a constant feature of the anuric case in which the blood pressure often rises during the anuric phase and *falls abruptly (especially the diastolic element) in the terminal* stages (Maegraith 1944) There appears to be little available information concerning the circulating blood volume in blackwater fever Lahille (1915) reported an increase (based on the relative volumes of serum *and clot) in one anuric case and Fairley and Bromfield (1934)* found a haematocrit value of only 6 8 per cent in a case in which the erythrocyte count was 0 9-5 million cells per cu mm These figures suggest that allowing for the possible fallacies of relying solely on haematocrit readings (Feldman and Murphy 1945) the circulating blood volume *in blackwater fever is certainly not always reduced and may* be increased It is thus unlikely that the anuria in blackwater fever can arise from general loss of blood volume

It has been pointed out by several workers that under many conditions variations in glomerular blood pressure may occur independent

factor in some cases of malaria and blackwater fever particularly if secretion of acid radicals directly through the cells takes place It does not explain however the appearance of epithelial lesions in cases secreting alkaline urine or in subjects suffering from alkalosis in whom identical lesions may develop (McLetchie 1943)

There remains the postulate that the epithelial changes are due essentially to local conditions of anoxia In the production of the lesions of the kidney as in all organs in malaria and blackwater fever there is a background of tissue anoxia arising from anoxaemia caused principally by the destruction of parasitized and unparasitized erythrocytes Such anoxia no doubt effects general changes in the epithelial cells of the whole nephron but it cannot easily account for the localization of the major changes in the convoluted tubules even supposing the development of lesions in these tubules were influenced in some cases by their specific function of adjusting the acidity of the urine Other factors must be concerned in the regional distribution of the lesions It will be noted that the epithelial changes occur chiefly in the cortical portions of the nephron and it has been pointed out elsewhere that at autopsy the cortex is frequently found to be pale and anaemic in relation to the medulla Some degree of cortical ischaemia may thus be present either associated with a general redistribution in renal blood flow or confined to the cortex the consequent tissue anoxia may account for the development of the lesions in the cortically disposed tubules The concentration of the epithelial damage in the distal convoluted tubules may be also emphasized in the event of generalized cortical ischaemia by a redistribution of blood flow resulting in a selective reduction of the blood supply to these tubules which is according to some authors partly non-glomerular (Smith 1939-40 Maegraith 1944)

There is no direct clinical evidence of ischaemia in the renal cortex in malaria and blackwater fever In the absence of positive evidence apart from the frequent clear-cut indications of tubular insufficiency the argument for the existence of cortical ischaemia must be based for the present on the results of animal experiments and on the close similarity between the clinical features and pathological changes found in the renal syndromes of malaria and blackwater fever and those seen in many other conditions of renal dysfunction developing in acute illnesses and in which the evidence pointing to renal ischaemia is highly significant These points will be discussed below It should however be noted here that the histological picture of tubular damage can be reproduced in animals by experimental interference with the renal

The epithelial lesions

It has been demonstrated above that the passage of haemoglobin in the urine is not specifically concerned either directly or indirectly in the genesis of the epithelial lesions in blackwater fever although these may be aggravated thereby It has been shown that (i) the presence of parasites is not necessary for the production of the epithelial changes (ii) malarial pigment *per se* is not a significant factor and (iii) there is no unequivocal evidence of the activity of malarial toxins' or parasitic metabolites Moreover the changes in electrolyte concentration and body water balance which may develop in these diseases appear to *be insufficient to evoke the epithelial damage and the depression in* hydrostatic blood pressure and diminution of circulating blood volume which occasionally occur are inadequate in most cases to account for the renal failure Some other explanation of the epithelial degeneration is therefore needed and of the remaining possibilities the most likely appear to be (i) that the cellular changes arise from a defect of function specific to the part of the nephron involved or (ii) that localized tissue anoxia is developed and gives rise to non-specific degeneration and necrosis

Many authors have stressed the severity of the epithelial degeneration in the convoluted tubules particularly in the distal tubules and in the ascending loop of Henle It is possible that some of this damage may be related to the specialized function of these portions of the nephron particularly in regard to the acidification of the urine It is generally agreed that the urine filtered off through the capsular membrane has the same pH as plasma (Wearn and Richards 1924 Ellinger 1934 1940) and that the filtrate undergoes a process of acidification in its passage down the nephron Experimental evidence in the frog and the rat indicates that if the ultimate acidity of the urine in relation to the plasma is slight the acidification takes place exclusively in the distal convoluted tubules whereas if the bladder urine is highly acid (for instance in animals given ammonium chloride) both the distal and proximal tubules are involved (Ellinger and Hirt 1929 Richards 1929 Ellinger 1934 a and b 1940 a and b) Ellinger (1940 b) has also shown that in rats given ammonium chloride acid urine is apparently secreted direct through the epithelium of both the proximal and distal tubules Dunn *et al* (1941) have suggested that this may be an important factor in the evolution of similar tubular damage seen in the experimental administration of large amounts of urates and phosphates (Dunn and Polson 1926 McFarlane 1941) and it may also be a contributory

factor in some cases of malaria and blackwater fever particularly if secretion of acid radicals directly through the cells takes place It does not explain however the appearance of epithelial lesions in cases secreting alkaline urine or in subjects suffering from alkalosis in whom identical lesions may develop (McLetchie 1943)

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the passage of pigment in the urine sometimes not The feature which distinguishes this syndrome from other forms of acute nephritis is its reversible nature (Maegraith *et al* 1945) The close resemblance between the renal symptoms met in blackwater fever and those of crush injury and incompatible blood transfusion has been commented on by many authors (Mayon-White and Solandt 1941 Bywaters and Beall 1941 Bywaters and Dible 1942 Bordley 1931 Kimmelstiel 1938 Foy *et al* 1943) Kimmelstiel reported the syndrome which he called acute haematogenous interstitial nephritis in certain acute conditions including lobar pneumonia and chemical and heat burns and drew attention to its appearance in infectious diseases septicaemia the hepatorenal syndrome and non bacterial food poisoning Tomb (194-) emphasized the similarity between the renal failure of cholera and that of the crush syndrome and Foy *et al* (1943) pointed out the common features of the azotaemia occurring in blackwater fever sulphonamide haemoglobinuria (Wood 1938) and in other conditions in which there is intravascular lysis of erythrocytes and where pigments were excreted in the urine such as crush injury incompatible transfusion and favism Maegraith (1944) Maegraith *et al* (1945) and Darmady (1947) have reviewed the literature and demonstrated that the syndrome has been described in *incompatible blood transfusion* (Witts 19-9 Bordley 1931 Ayer and Gauld 1942) *crush injury* (Husfeldt and Bjering 1937 Longland and Murray 1941 Henderson 1941 Mayon-White and Solandt 1941 Dunn Gillespie and Niven 1941 Bywaters and Beall 1941 Bywaters and Dible 194-) *injuries* associated with shock *traumatic uraemia* (Darmady *et al* 1944 Parsons 1945 Darmady 1947) *surgical shock* (Moon 1944) the *hepatorenal syndrome* (Schutz *et al* 1932 Boyce and McFetridge 1935) *septic abortion and concealed accidental haemorrhage* (Bratton 1941 Young 1942) *cholera* (Rogers 19-1 Chatterjee 1941 Tomb 194-) *yellow fever* and *Weil's disease* (Stokes *et al* 1917 Hoffmann 1924 Beewkes 1936 Findlay 1941 Harris 1942 Wylie 1947 Williams 1947) and *alkalosis* (Ellis 19-4 Nicol 1940 McLetchie 1943) A somewhat similar syndrome has also been described in *pernicious anaemia* and *mercury* and *bismuth* poisoning (Fishberg 1939)

Maegraith (1944) suggested that these renal syndromes all had a common basis and grouped them under the term tubulovascular renal syndrome Later he and his colleagues (1945) reviewed the evidence and showed that the basic aetiological factor was probably anoxia of the renal tissue particularly the cortex and proposed the more appropriate term renal anoxia Lucke (1946) has recently

blood flow (Scarff and Keele 1943) and that the intravenous injection of haemoglobin has been shown to bring about diminution in renal volume arising from vascular constriction and reduction of blood flow (Mason and Mann 1931) and in some cases reduction of glomerular blood flow in relation to total flow (Corcoran and Page 1945)

The development of anuria

Anuric cases of malaria and blackwater fever are almost always associated with pronounced degenerative changes in the epithelium of the convoluted tubules and with plugs of debris in the lumina and as we have seen many authors have attempted unsuccessfully to define a causal relation between the failure of urinary flow and the tubular lesions and apparent obstructions It has been shown elsewhere that there is no evidence of increased reabsorption of fluid in anuric cases and that anhydraemia arising from tissue dehydration cannot be considered to be an operative factor in all cases although it may influence urinary flow in some cases in which fluid has been lost by vomiting or diarrhoea or in which the electrolyte-water balance of the plasma has been grossly upset Depression of hydrostatic blood pressure and diminution in circulating blood volume have also been shown to be inconstant features and often absent at the onset of anuria It appears therefore that anuria developing in malaria and blackwater fever is like that appearing in many other conditions the result of deficient glomerular filtration which as Black *et al* (1941) showed in connection with anuria following severe haematemesis can be best explained by a reduction in plasma flow to the filtering surface i e a reduction in glomerular blood flow Apart from obvious oliguria anuria and signs of water retention there is little direct evidence in blackwater fever or malaria of reduced glomerular filtration It is thus unfortunately necessary again to argue by analogy first by establishing that the anuric syndromes of these diseases are identical with those seen in other conditions and second by demonstrating that in the latter there is strong evidence of reduced glomerular filtration and blood flow Such arguments are justly open to criticism but until evidence one way or the other is presented they are the only means of analysing the renal syndromes under discussion and serve to provide a basis for rational experiment

The syndrome of tubular dysfunction complicated by anuria nitrogen retention and degenerative changes in the epithelial cells of the convoluted tubules has frequently been reported in many acute illnesses besides malaria and blackwater fever sometimes associated with

Maegraith *et al* (1945) pointed out however that many of these hypothetical toxins have not been identified and that they must all be assumed capable of producing syndromes which are similar in their clinical and pathological appearances

De Gowin *et al* (1938) mentioned that Russian workers favoured the view that renal insufficiency in incompatible transfusion arose from ischaemia of the kidneys and Kimmelstiel (1938) after noting the similarity of the renal symptoms in a large number of conditions pointed out that general or local impairment of the renal circulation probably occurred in all of them In cases in which the peripheral circulation failed and the blood pressure fell the capillary blood pressure in the glomeruli was lowered and reduced filtration resulted and led to oliguria or anuria failing circulation gave rise to diminution of the oxygen supply to the epithelium of the tubules and consequent derangement of their function evidenced by decreased concentration of the urine In cases where there was no obvious peripheral circulatory collapse Kimmelstiel suggested that there was still impaired renal circulation resulting either from tissue oedema or vascular spasm He stressed the view that anuria and tubular dysfunction both resulted from general or local circulatory disturbances and were coincident rather than causally related

Bywaters and Dible (194-) in a careful account of the renal syndrome and pathological changes occurring in crush injury concluded that mechanical blockage was not the sole agent responsible for failure of urinary flow and suggested that this arose either from diminution of glomerular filtration due to reduction in (glomerular) blood pressure or blood flow or from excessive but unselective reabsorption of the glomerular filtrate through the tubules They supported the latter view

Moon (1944 194- 1938) considered that the renal changes developing in shock and traumatic injury resulted from anoxaemia of the kidneys contingent upon the liberation of H substance from the damaged tissue This anoxaemia (which presumably arose from reduced renal circulation) led to oliguria and azotaemia as well as the tubular degeneration Experimental evidence of the production of such changes in the kidneys following histamine injection was obtained by Hashimoto (19-5)

Maegraith *et al* (1945) exhaustively reviewed the literature and concluded that the most satisfactory explanation of the appearance of the syndrome was the development of anoxia of the renal tissue arising from reduced renal blood flow affecting mainly the cortex and asso-

classified the syndrome as lower nephron nephrosis It has been suggested above that the tubular deficiency developing in malaria and blackwater fever arises from tissue anoxia induced by cortical ischaemia and that accompanying anuria results from gross reduction of plasma flow at the filtering surface of the glomeruli and thus indicates a diminution of glomerular blood flow Laboratory information concerning renal function is unfortunately lacking in malaria and blackwater fever but evidence supporting both these contentions is available in many of the examples of the renal anoxia syndrome quoted above and in similar syndromes experimentally induced in animals Such evidence may be taken to apply to some extent to malaria and blackwater fever if the reasonable assumption be made that the renal syndromes seen in these conditions are identical with those grouped within the term renal anoxia and are caused by similar pathological processes

Renal blood flow in renal anoxia

Many theories have been advanced to account for the tubular damage and failure of urinary flow in various examples of the renal anoxia syndrome In those conditions in which pigment is passed in the urine the mechanical blockage hypothesis has frequently been strongly supported This suggestion has been discussed fully elsewhere in this Chapter (page 227) where it has been shown that it is now generally agreed that mechanical obstruction of urinary flow arising from tubular blockage cannot be more at most than a contributory factor in the genesis of the renal failure (Bywaters and Dible 104- Maegraith *et al* 1945 Darmady 1947)

Experimental crushing of limbs or injection of myoglobin or haemoglobin in animals have not been found to reproduce the renal syndrome unless the subjects are in a state of acidosis (Bywaters and Popjak 1942 Eggleton *et al* 1943 Bywaters and Stead 1944 Yule *et al* 1945) Foy *et al* (1943) have suggested that the factors involved in the development of the syndrome in some cases associated with circulating pigment are dehydration and reduction in blood volume and renal circulation A nephrotoxic effect produced by some noxious substance conveyed to the kidney by the blood stream or resulting from changes in electrolyte concentration of the plasma has often been postulated as the cause of the syndrome in intravascular haemolysis (in which the circulating pigment has been implicated) crush injury hepatorenal syndrome and various infections such as cholera and Weil s disease (Bordley 1931 Bywaters and Dible 1942 Eggleton *et al* 1943 Eggleton 1944 Schutz *et al* 193- Stieglitz 1924)

proportional reduction of the inulin and diodrast clearances but in the other eight the former clearance was reduced more in proportion (as much as 40 per cent) than the latter These results indicated a differential reduction in glomerular flow

The reduction in total plasma flow recorded in some of Page s animals following haemoglobin injection is consistent with the findings of other authors mentioned elsewhere who demonstrated a diminution in renal volume in similar circumstances (Mason and Mann 1931 Hesse and Filatov 1933)

Scarff and Keele (1943) showed that degeneration of the renal tubules resembling that seen in crush injury could be produced in rabbits by temporary total occlusion of the renal blood flow for periods of up to three hours Animals which survived this operation for some days developed azotaemia The kidneys showed degenerative changes in the tubular epithelium especially in the first portion of the secreting tubules granular and hyaline casts were present in the distal convoluted and collecting tubules The glomeruli were unaffected The authors suggested that the kidney lesions of crush syndrome might be due to renal ischaemia which was not as complete as that developed in the animals by clamping the renal artery but which acted for a longer time Evidence of such diminution of renal flow has been provided by Black *et al* (1941) who found a reduction of perabrodil clearance in such cases Scarff and Keele point out that changes in renal blood flow may occur independently of any change in systematic blood pressure Darmady (1947) states that van Slyke *et al* (1944) and Badenoch and Darmady (1947) have obtained similar results to those of Scarff and Keele

Yule *et al* (1945) summarized the literature on the genesis of renal failure associated with intravascular haemolysis and noted that all such syndromes have in common a circulating pigment a factor of vascular or chemical nature affecting the organism as a whole and a specific renal lesion They examined the effects of injection of haemoglobin with and without concomitant temporary clamping of the renal artery or poisoning with sodium tartrate in rabbits secreting acid and alkaline urines They confirmed Scarff and Keele s results and showed that haemoglobin injection gave rise to most serious consequences in those animals which had had their renal tubules damaged by ischaemia or tartrate The maximum degree of tubular injury occurred in aciduric animals

Measurement of renal clearances in human cases has yielded good evidence of disturbances of kidney blood flow during the course of the

ciated in many cases with general peripheral vascular collapse They later (1946) elaborated this hypothesis and suggested that the available evidence pointed to there being in all cases whether associated with general peripheral vascular collapse or not a reduction in total renal blood flow and an intrarenal redistribution of blood flow whereby the cortex is rendered especially anaemic and thus anoxic Maegraith and Findlay (1944) had previously postulated a cortical anaemia in blackwater fever resulting from the shunting of the blood flow from the cortex to the medulla They considered that cortical ischaemia and its attendant anoxia initiated the tubular degenerative changes and that anuria developed from a specific failure of glomerular flow

This vascular conception of the pathogenesis of the renal anoxia syndrome has been challenged on general grounds with regard to the renal syndrome seen in alkalosis (McCance and Black 1946) Nevertheless it is well supported by experimental evidence The syndrome can be very closely reproduced in animals under certain conditions by injection of lithium monourate or sodium acid phosphate (Dunn and Polson 1926 McFarlane 1941) by severe haemorrhage (Corcoran and Page 1943 Phillips *et al* 1946) and by injection of haemoglobin and myoglobin into aciduric subjects

Corcoran and Page (1943) investigated the diodrast (total plasma flow) and inulin (glomerular flow) clearances and changes in urinary volume in dogs during hypotensive states initiated by severe haemorrhage and again after transfusion During the hypotensive phase there were marked changes in both clearances and a considerable reduction in urinary output They concluded that the renal flow during hypotension is distributed *irregularly* through the renal vascular bed The same authors (1945) carried out more detailed experiments in unanaesthetized trained dogs in a study of the effects of injections of haemoglobin myoglobin and sodium ferrihaemate They came to the conclusion that injection of myoglobin and haemoglobin produced partially recoverable renal injury of varying degree and that myoglobin might be a significant factor in the pathogenesis of the renal syndrome in crush injury Experiments in rats indicated that other factors including a state of dehydration were also important In some ways their interpretation of their results was unsatisfactory For instance they failed to comment on their own clear demonstration of a differential redistribution of renal flow following the injection of pigment In experiments in which the immediate effect of injection was measured diodrast clearances were increased in five animals unchanged in one and reduced in four In two animals there was a

In discussing the pathological picture seen in the kidneys in malaria and blackwater fever it was pointed out that the cortex was often pale and anaemic and the glomeruli as a rule either normal or anaemic the medulla was deeply or irregularly congested and there were scattered tubulovascular haemorrhages Similar changes have been described in crush injury incompatible transfusion traumatic uraemia and other examples of the renal anoxia syndrome (Witts 1929 de Navasquez 1940 Bywaters and Dible 1942 Darmady 1947) Histological evidence of this sort is in itself difficult to correlate with functional derangements but it lends support to the evidence outlined above in favour of the view that in these examples of the renal syndrome there is some degree of cortical ischaemia involving especially the glomeruli

Information regarding inulin and diodone clearances in malaria and blackwater fever is lacking but if it be agreed that the renal syndromes seen in these diseases are basically similar to those included in the renal anoxia group the available evidence histological experimental and clinical indicates that there is a diminution of total renal flow and that in anuric cases there is a further reduction in glomerular flow and corresponding failure of filtration Reduction of urinary flow is more satisfactorily explained on this basis than on increased intra renal pressure or excessive unselective re-absorption of the glomerular filtrate by the tubules (Paramore 1945 Peters 1945 Dunn *et al* 1941 Bywaters and Dible 1942)

It has already been noted that the destruction of erythrocytes and other changes in the blood lead to anoxaemia in malaria and blackwater fever The effects of this on the tubules would be exaggerated by a reduction of the cortical blood flow particularly in those areas which have the poorest blood supply or in which the secretory activity is great (Dunn and Polson 1926 Macgraith 1944 Darmady 1947) The anoxaemia and reduced blood supply together would lead to local anoxia and consequent damage to the epithelium Fishberg (1939) believed that a mechanism of this sort was involved in the production of the defective renal function seen sometimes in pernicious anaemia which he considered arose from poor nutrition of the kidney cells from the anaemic blood The production of tubular epithelial lesions by obstruction of the renal artery has already been discussed Impairment of tubular function has also been experimentally obtained by haemorrhage provided the blood haemoglobin is reduced sufficiently (Corcoran and Page 1943 Essen and Porges 19..) Carbon monoxide poisoning in which the supply of oxygen to the tissues is considerably

renal anoxia syndrome Cournand *et al* (1943) and Lauson *et al* (1944) demonstrated very considerable reduction in renal blood flow in oliguric cases of peripheral circulatory collapse following severe non-crushing injuries They found that the fall in diodrast clearance was more than could be accounted for by the lowering of systemic blood pressure or general circulatory rate and was best explained by the presence of increased vascular resistance (vasoconstriction) within the kidney They concluded that their investigations confirmed the view that the urinary findings in shock i e oliguria or anuria and loss or impairment of concentrating power are the result of decreased circulation through the kidney Richards (1944) supported this hypothesis

Black *et al* (1941) studied the clearances of inulin and perabrodil in cases of azotaemia occurring after severe haematemesis They observed a similar reduction of total renal flow and a greater fall in glomerular filtration which they attributed as previously stated to diminution of the plasma flow across the filtering surfaces of the glomeruli i e to a reduction in glomerular blood flow the latter being due to changes in the systemic blood pressure circulation and circulating blood volume associated with shock

The renal disturbances resulting from high dosage of alkali (which in their severest form amount to the established syndrome of renal anoxia) were investigated by members of the Army Malaria Research Unit (1945) who determined inulin diodone and urea clearances in subjects taking a mixture of sodium bicarbonate and citrate The alkali was administered for a maximum of three days and water was taken freely during the experiments so that urinary volume was well maintained After the first few doses the glomerular filtrate increased considerably although the total renal plasma flow remained unchanged The filtration fraction estimated as the ratio of the inulin to the diodone clearances was thus raised it remained high during the rest of the experiment towards the end of which the inulin clearance returned to within normal limits and that of diodone was reduced Clinical signs of water and sodium retention were present by the conclusion of the alkali administration and in one subject the blood urea nitrogen rose appreciably and urea clearance was greatly reduced Recovery was rapid after alkali was stopped The results of these experiments were interpreted as showing the development of transient injury to the tubular epithelium and a redistribution of the intrarenal blood flow whereby the glomerular flow was increased in proportion to the total flow

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reduced may also give rise to renal symptoms including tubular insufficiency (Herzog 1920 Drinker 1938) Diminution of urinary secretion in dogs exposed to low atmospheric oxygen pressure was observed by van Liere *et al* (1935) and by Toth (1940) who found that the oliguria produced in such circumstances could be abolished by denervation of the kidneys but was unaffected by adrenalectomy Exposure of dogs to anoxic anoxia however occasionally led to polyuria which has also been observed in human subjects exposed to slight reduction in atmospheric oxygen No information is apparently available concerning the changes of the renal function in mammals exposed to severe anoxic anoxia but in frogs complete absence of oxygen leads to anuria due to cessation of glomerular flow following constriction of the renal arterioles (Adolph 1934)

Changes in renal blood flow

Maegraith and Findlay (1944) and Maegraith and Havard (1946) suggested that the lesions in blackwater fever resulted from by-passing of the cortical blood flow mainly to the medulla and partly to the subcapsular plexus They considered that in addition to reduction of total renal plasma flow an active redistribution of intrarenal blood flow took place increasing the cortical ischaemia especially at the expense of the glomeruli These differential adjustments of blood flow within the kidney might occur through specific vascular channels or as the result of active variation in the calibre of the vessels concerned

The manner in which the latter can be initiated is indicated by recent experimental work Lauson *et al* interpreted their renal clearance findings in traumatic shock in terms of increased vascular resistance (constriction) and Darmady *et al* (1944) held that the general reduction in renal flow in traumatic uraemia resulted partly from prevailing low blood pressure and partly from intrarenal vascular spasm

Trueta (1945) in a note referring to the hypothesis of Maegraith *et al* (1945) suggested in the light of experiments he performed with Barnes in which a widespread arterial spasm was initiated in the body by unilaterally applied trauma to a limb that the renal vascular constriction in traumatic uraemia was reflex in origin and resulted from overstimulation of peripheral nerves Evidence indicating that renal vasoconstriction might in fact be initiated by afferent stimuli arising in peripheral nerves was brought forward earlier by Andrews (1027) who demonstrated oliguria and anuria in dogs following the release of tight ligatures of the limbs and by Bieter (1930) who confirmed previous work showing that stimulation of the central ends of

severed peripheral nerves including the sciatic led to vasoconstriction in the kidneys in frogs

A powerful nervous control of the renal blood vessels in animals has been frequently demonstrated (Bradford 1889 Hirt 1926 Kuntz 1929 Bieter 1930 Harris and Harris 1931 Milles *et al* 1931 a and b 1932 Smith 1939–40) For instance direct stimulation of the renal or splanchnic nerves brings about intrarenal vasoconstriction and section of the splanchnics results in an increase in intrarenal blood flow It has been shown by various methods including the study of renal clearances that under normal conditions the renal blood flow in man is controlled mainly by alterations in the bore of the efferent arterioles of the glomeruli in such a way that the urinary filtration rate through the glomeruli is kept constant (Smith 1937 1939–40) Direct or reflex stimulation of the vasomotor nerves (apparently all vasoconstrictor) coming from the renal sympathetic plexus leads to constriction of the efferent vessels When the stimulus is excessive e g after large doses of adrenalin extreme degrees of renal ischaemia may develop owing to constriction of the afferent as well as the efferent vessels

Reflex vasoconstriction of the renal vessels may apparently arise from intrarenal as well as extrarenal stimuli such as those referred to above Maegraith and McLean (1938 1942) demonstrated this indirectly in experiments in rabbits in which the appearance of Goldblatt (1937) hypertension (the production of which requires a minimum of 40 per cent reduction in renal blood flow) was used as an indication of reduced renal flow They showed that slight damage to the kidney tissue induced by injection of diatomaceous particles gave rise to reflex vasoconstriction which could be prevented or abolished by section of the renal nerves

If as suggested stimulation of vasoconstrictor nerves is responsible for the development of the renal ischaemia which gives rise to the renal anoxia syndrome breaking the reflex path should relieve the syndrome The relief of anuria in some cases of reflex anuria traumatic anuria and Weil s disease by manoeuvres such as splanchnic block or high spinal anaesthesia has been explained on this basis (Darmady 1947 Williams 1947 Hesse and Filatov 1933) Too much reliance cannot safely be placed on such clinical results however since the renal anoxia syndrome is fundamentally reversible and recovery may take place in some cases without treatment of any sort Nevertheless the balance of evidence is in favour of the value of such treatment and this taken in conjunction with the evidence of vasoconstriction and changes in renal blood flow already discussed and the interesting results of

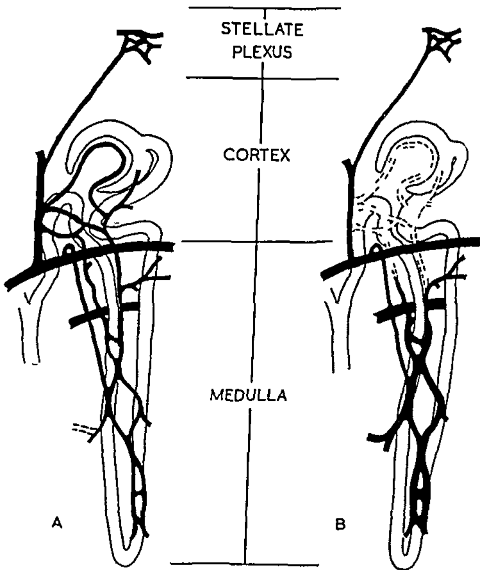


Chart 6—Di g mm tic ep esent tion of the eff t of co tical isch on glomerul r blood flow Vessels in bl k Neph on haded

A Normal flow

B Glomerular blood flow by passed to stell te ple s and medulla As explain d in the t xt it is p obabl that a red tribution of th c al blood flow o curs in the re l anoxia syn'l ome wh ch ppe s in seve m l ria a d bl ckw t f r

Toth on the effects of renal denervation in experimental anoxic anoxia indicates that renal ischaemia may arise from reflex vasoconstriction initiated by either extra- or intrarenal stimuli The great reduction in intrarenal blood flow occurring in many examples of the renal anoxia syndrome can be explained in this way but general renal ischaemia cannot be held to account for the apparently specialized failure of glomerular flow in anuric cases

Trueta *et al* (1946 1947) have thrown some light on this problem recently by demonstrating in animals a reflex mechanism capable of differentially diverting the blood flow from the renal cortex to the medulla much as was suggested by Maegraith and Findlay (1944) in blackwater fever Their observations were confined to the results of extrarenal stimulation of the renal nerves and are therefore applicable only to the development of cortical ischaemia in conditions such as traumatic injury in which such extrarenal excitation is likely It is possible however that this reflex adjustment of the circulation in the kidney can also be initiated by intrarenal stimuli and may explain the results of Maegraith and McLean already quoted above and the appearance of cortical ischaemia in examples of the renal anoxia syndrome in which general vascular collapse is not a characteristic feature

Shunting of the blood from the cortex to the medulla takes place according to Trueta *et al* through the true *arteriae rectae* and possibly as suggested by Maegraith and his colleagues (1944 1946) through other non-glomerular channels including the arteriovenous anastomoses described by Steinach (1884) and Spanner (1938) in the pelvis cortex and subcapsular plexus which according to Smith (1939–40) afford a mechanism for the direct perfusion of the tubules at a time when the glomerular circulation is largely arrested Heggie (1946) has challenged this view and pointed out that most of the work prior to that of Trueta *et al* tended to disprove the existence of a non glomerular medullary blood supply He suggested that the changes in renal flow which developed in Trueta s experiments and which were discussed by Maegraith and his colleagues in the renal anoxia syndrome could arise from the maintenance of circulation in the deep juxtamedullary glomeruli at a time when the rest of the cortex was anaemic following vasoconstriction of the peripheral arterial vessels in response to afferent stimuli He held that the wide efferent vessels coming from these deep glomeruli could maintain the circulation sufficiently without requiring the existence of either vasa recta or the arteriovenous shunts of Spanner and Steinach Heggie (1947) in a subsequent

note states that material provided by the Oxford group demonstrates the importance of the efferents of the deep glomeruli His general argument regarding the doubtful existence of a non glomerular circulation in the kidney is however not completely satisfactory since it does not take into account such experimental results as those of Maegraith and McLean (1939) who demonstrated that the frequently observed spontaneous resolution of Goldblatt hypertension (initiated by clamping the renal artery in rabbits) resulted from the restoration of normal renal blood flow by the establishment of collateral circulation through the renal capsule from the perirenal vessels It is difficult to see how such collateral circulation could develop within the kidney except through non-glomerular vessels connecting the subcapsular plexus with the main renal circulation The evidence provided by Trueta and his colleagues is very convincing so far as the demonstration of the production of cortical ischaemia following stimulation of the renal nerves is concerned The exact vascular channels through which the cortical blood flow is diverted in this reflex cannot yet however be said to be finally demonstrated

RECAPITULATION

CORTICAL ISCHAEMIA AND RENAL ANOXIA IN MALARIA AND BLACKWATER FEVER

The pathogenesis of the renal syndromes of malaria and blackwater fever appears to be fundamentally identical and similar to that of like syndromes arising in many other conditions listed as examples of renal anoxia The tubular lesions in such syndromes can best be explained on a basis of tissue anoxia and the anuria (and associated azotaemia) by reduction in glomerular filtration leading to cessation of urinary flow The arguments discussed above serve to show that the most rational explanation of these phenomena is the development of a change in intrarenal blood flow which brings about cortical ischaemia and reduction in glomerular blood flow and consequently in glomerular filtration The available evidence points to the existence of a renal vasoconstrictor reflex which can give rise to cortical ischaemia and can be initiated by both intra- and extrarenal stimuli

The following will illustrate the way in which the syndrome probably arises

In malaria there is general anoxaemia and tissue anoxia arising from (i) loss of both parasitized and unparasitized erythrocytes (ii) loss of haemoglobin on conversion into haemozoin (iii) removal of oxygen

from oxyhaemoglobin in parasitized cells by the parasites and (iv) intravascular agglutination There is also possibly histotoxic anoxia arising from interference with dissociation of oxyhaemoglobin and fixation of the oxycytochrome systems in the tissues perhaps by some product of the metabolism of the parasite Finally there are changes in the endothelial walls of the blood vessels which lead to local escape of fluid and slowing of the circulation through the small vessels

Tissue anoxia leads to changes in the renal tissue and affects both the endothelium of the blood vessels and the epithelium of the tubules

As a result of this the intrarenal reflex is stimulated and changes in the circulation take place leading to some degree of cortical ischaemia and the anoxic state of the epithelial tissues becomes aggravated Changes in the cortical vessels greatly reduce glomerular flow and the output of urine falls the patient finally becoming anuric In severe cases of malaria blackwater fever etc general circulatory changes of the nature of shock with reduced circulating blood volume and low blood pressure supervene and the extrarenal reflex is also activated

In the early stages of the syndrome in malaria therefore the intrarenal stimulus is the important one In other conditions e g shock the extrarenal reflex is probably the initiating factor In shock and similar general conditions there is evidence to show that the extrarenal reflex is only part of a bodily protective mechanism which involves other organs including the adrenals and the liver

CHAPTER IX

THE BRAIN

CLINICAL PATHOLOGICAL CHANGES Macroscopic appearances — Histological appearances General picture Parasites Phagocytos Changes in blood vessels Vascular obstructio Haemorrhages Degenerative changes in brain cells Neuroglial changes, granulomata PATHOGENESIS Vascular obstruction — Stasis — Effects of heat — Anoxia

CLINICAL

THE neurological manifestations of malaria are protean Clinical *evidence of involvement of the brain and spinal cord is most com*monly seen in *P falciparum* infections but may occur in other forms of malaria The presenting signs and symptoms can sometimes be related to causal lesions which may be purely temporary or of a more permanent nature depending on the development of structural changes e g following haemorrhage into the brain substance In many cases however there is no obvious relation between the pathological state of the brain and the clinical features of the individual case Neurological symptoms often occur in fatal cases with no localizing lesions and changes in the brain may not be accompanied by any related clinical phenomena

There are many reviews on the subject of the neurological manifestations of malaria and these should be consulted for further details Thomson and Annecke (1926) divide the nervous signs of malaria into those associated with acute malaria and those associated with chronic

The signs in acute malaria include headache irritability the cerebral state (see Chapter I) hyperpyrexia uncontrolled sweating apoplectic and epileptiform attacks and convulsions frontal lobe syndromes (Brill and Pellicano 1943) bulbar paralysis cerebellar disturbances meningeal symptoms polyneuritis (Lafora 1912) tremors in chronic malaria multiple sclerosis (de Vries 19-7) peripheral neuritis and amaurosis (Kitchen 1941)

There is also often very clear evidence of mental disturbances including delirium coma amnesia motor aphasia and various types of psychoses neurasthenia confusion and depression sometimes developing into dementia praecox and delusional insanity (Masson 19-4 Forrester 1920 Carhill 1917 Browne Mason 1905 Arieti 1946 Pasmanik 1897)

PATHOLOGICAL CHANGES

Macroscopic appearances

The information given below has been culled almost entirely from accounts of autopsy findings in cases of *P falciparum* malaria or blackwater fever In the few cases where examinations have been made in cases of other forms of human malaria the general appearances and lesions are much the same (Billings and Post 1915 Marchiafava and Bignami 1900)

Meninges Some authors have reported *no* abnormal changes in the meninges (Marinesco 19_1) Others have described various degrees of congestion involving most of the vessels especially those of the pia mater There may be small haemorrhages usually petechial sometimes as large as half an inch in diameter and occasionally localized in certain areas e g in the pia covering the cerebellum or cortex (Rigdon 1942 Gaskell and Millar 19_0 Thomson and Annecke 19_6) Infiltration with round cells thickening and oedema of the pia have also been described (Thomson and Robertson 1929 Margulis 1914) True leptomeningitis has been reported by Durck (1917 19_5)

Brain and spinal cord Marchiafava and Bignami (1900) described the leptomeninges and cerebral substance in cases of cerebral pernicious malaria as intensely hyperaemic They considered that the combination of such hyperaemia and dark brown or blackish pigmentation of the brain substance (due to the presence of malarial pigment) was practically diagnostic of malaria Most workers are agreed that the brain surface is hyperaemic and covered with engorged vessels (Dudgeon and Clarke 1917 Arieti 1946) even when the patient has clinically not suffered from cerebral symptoms The congestion and hyperaemia is not confined to the cerebral hemispheres but is usually seen over the whole brain and sometimes the cord Occasionally certain regions appear more affected than others e g the cortex in the vertex region (Gaskell and Millar) or the cerebellum (Marinesco 19_1) Accounts of pigmentation vary from not often pigmented externally (Craig 1909) to grey slate grey smoky grey or brown (Thomson and Annecke 19_6) Oedema of the brain substance has been described (Thayer 1899 Thomson and Annecke 19_6 Margulis 1914 Arieti 1946) The surface may be spotted with haemorrhages usually minute but sometimes of considerable size (Thomson and Robertson 19_9)

The cut surface of the brain substance is hyperaemic and the small vessels bleed freely Punctate haemorrhages are scattered freely about

the substance most commonly in the white matter but sometimes also in the grey (Arieti 1946)



Fig 15—Macroscopic appearance of brain in acute severe malignant tertian malaria Note distribution of haemorrhages (After Ash and Spitz 1946)

In deeply pigmented brains the cut surface is slate grey or brown in colour the deeper shades being found in the grey substance of the cortex and ganglia The brain substance is intensely hyperaemic and the surface dotted with minute bleeding points where the small vessels have been cut Scattered freely about in the whole substance of the brain and occasionally in the cord (Rigdon 1944) there may be small punctate haemorrhages Occasionally there may be large haemorrhagic areas Gaskell and Millar found no haemorrhages in

the grey matter although in their cases of cerebral malaria (*P falciparum*) they were present in the white substance of the corona radiata of the internal capsule and the caudate and lenticulate nuclei Haemorrhages have also been reported in the corpus striatum and cornu ammonis (Marinesco) They are most common in the white substance near the cerebral cortex and may be absent from deeper parts of the brain although abundant in this region For example in Gaskell and Millar s cases there were no haemorrhages in the pons and medulla although they were abundant in the subcortical white substance They may be present in the white substance of the cerebellum (Rigdon 1942) They have been reported in the grey matter of the cerebral cortex and are frequently found in the boundary zone between the cortex and the white substance (Marchiafava and Bignami 1900 Thomson and Annecke 1926 Arieti 1946)

Apart from the pigmentation and hyperaemia of the brain tissue and the scattered punctate haemorrhages there are usually few other changes visible macroscopically in the brain in malaria The lateral ventricles may be dilated and sometimes filled with blood stained fluid The choroid plexus has been found congested Irregularly distributed areas of softening have also occasionally been described (Craig 1909 Margulis 1914) The larger blood vessels were found to be free from evidence of thrombosis in the cases described by Gaskell and Millar (1920)

As will be seen later the changes described above are frequently seen in cases with no clinical history of cerebral malaria as well as in those with obvious neurological symptoms Occasionally in patients with clear clinical evidence of central nervous system disturbances there may be strikingly few changes in the brain at autopsy In some pernicious cases particularly those associated with vascular collapse e g algid malaria the brain may be anaemic in appearance and not congested or hyperaemic Under such circumstances there are usually few parasites in the brain capillaries In blackwater fever the brain may be pale with or without haemorrhages into the substance (e g into the cerebellum pons and corona radiata) and parasites are not commonly present in the vessels (Whipple 1909)

Histological appearances

General picture

The most striking features of the histological picture of the brain in severe malaria are the dilatation and congestion of the small blood

vessels and the associated haemorrhages The capillaries are usually loaded with red cells almost all of which may be parasitized The larger vessels contain fewer parasites which are often distributed around the periphery Sometimes there may be very few parasites in the small vessels and the red cells become clumped together into practically homogeneous masses in which the individual cells can no longer be distinguished *In some brains the distribution of parasitized cells* may be largely regional some vessels being packed with them others being free or relatively free of them Evidence of damage to the endothelial lining walls of capillaries and small arterioles is common There are frequently small perivascular haemorrhages suggestive of diapedesis of cells through the walls of the vessels and in many areas mainly in the white substance especially in the subcortical layers of the cerebral hemispheres there are also frank haemorrhages with consequent disruption of the local brain tissue The red cells of the haemorrhages are usually non-parasitized Scattered through the brain substance there may be nodules of degenerate tissue and red cells surrounded by accumulations of small glial cells and often with a centrally placed small arteriole These nodules are the so-called malarial granulomas and are by no means constant features

Degenerative changes in the neurological tissue are common especially in regions affected by haemorrhage or granulomatous reactions Swelling and vacuolization of the cytoplasm loss of Nissl granules and necrotic changes in the nuclei of nerve cells may be present Degeneration of medullated fibres also occurs

Cellular reactions in the form of accumulations of small round glial cells in the brain substance sometimes wide spread and lymphocytes and plasma cells in the perivascular spaces are found in many cases especially in cases of long standing Such perivascular lymphatic accumulations are also met in the meninges Regeneration and repair processes are not common beyond some degree of replacement of areas damaged by haemorrhage Some authors have described irregular new formation of vessels in acute malaria

Parasites

If a small portion of brain substance be removed from the cerebral region at autopsy and examined after squashing between two glass slides parasites many of them pigmented will usually be found within the red cells filling the smaller blood vessels In many cases practically all the erythrocytes may be invaded In some there may be very few Gaskell and Millar have stated that in cerebral malaria

the greatest accumulations of parasites are to be found in the vessels of the grey matter although the pathological lesions are chiefly found in the white substance The parasites may be distributed evenly through the vessels of the brain or may occur irregularly sometimes being present in some and absent in other capillaries in the same region There may also be a regional distribution e g Margulis observed in one case a concentration of parasitized cells in the vicinity of the cortical ganglion cells Parasites may appear in every stage of development from the merozoite to the rupturing schizont Occasionally one particular stage of the life cycle predominates the prevailing form to some extent determining the pigmentation of the organ Thus when mainly young forms are present there is little pigment when on the other hand schizonts predominate there may be very heavy pigmentation Marchiafava and Bignami (1900) state that in some cases it may be possible to recognize almost every stage of parasite in the asexual life cycle within one individual capillary The erythrocytes within the capillaries contain the more mature forms of parasite as a rule The arterioles and larger vessels contain younger forms including young rings In the capillaries the parasitized cells are closely packed and may present the appearance of thrombosis The tightly packed cells are however probably not thrombosed but in a condition of stasis In the larger vessels the parasitized cells are usually fewer and often tend to be distributed along the vessel wall the cells in the centre of the lumen being non parasitized Marchia-

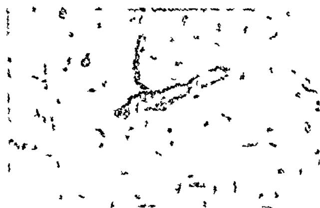


FIG 16—B[illegible] [illegible] ub[illegible] [illegible] se of m[illegible] [illegible] [illegible] wh[illegible] h[illegible]ther [illegible] [illegible] [illegible] mpt [illegible] th[illegible] [illegible] the [illegible] [illegible] th[illegible] [illegible] erythrocytes and th[illegible] [illegible] [illegible] of p[illegible] [illegible] th[illegible] [illegible] wall of th[illegible] l[illegible]g[illegible] vessel

fava and Bignami suggested that the presence of the bulk of parasitized cells in the small vessels is an indication of their increased resistance to flow through narrow channels compared to normal erythrocytes



Fig 17—Para ites in cerebral cap ll nes in a case of m l g a t tertian malaria Note the sporul t on of n tu e schizonts

Gaskell and Millar (19-0) commenting on the enormous numbers of parasites observed in their cases of true cerebral and septicaemic malignant tertian malaria suggested that in the former the brain may assume the function of the spleen in becoming a factory for the development of the parasite in all its forms Agonal development of parasites within the cerebral vessels continuing after circulation had stopped might explain the frequently observed predominance within the capillaries of malarial forms representing one stage of the life cycle

Most authors agree that the red cells accumulated in the tissues around the small vessels and those in frank haemorrhages are usually non-parasitized or very sparingly parasitized even when the associated vessel lumen is itself packed with parasitized cells (Marchiafava and Bignami 1900 Lafora 1912 Thomson and Annecke 19-6) Gaskell and Millar (19-0) however have reported the presence of both rings and crescents in such haemorrhages in malignant tertian infections

Parasites are often found in the vessels of the meninges especially the pia sometimes associated with apparent thrombosis or stasis (Gaskell and Millar 19-0 Thomson and Annecke 19-6 Margulis 1914) They are usually present in small numbers compared with those present in the brain itself

Phagocytosis

Pigmentation of brain tissue is usually associated with the presence of parasites in large numbers in the small vessels but some workers have reported free pigment lying in the blood vessels and in the brain substance itself sometimes in relation to haemorrhages or granulomatous nodules (Gaskell and Millar 19_0 Margulis 1914) Pigment is also frequently found within phagocytic cells lying free or within parasitized cells Phagocytosis of pigment and debris by the endothelial lining of the capillaries has also often been described Marchiafava and Bignami found these cells to be swollen and laden with pigment and Craig (1909) Thomson (19_4) Thomson and Annecke (19_6) and Seyfarth (1926) all report active phagocytosis by the endothelium Dudgeon and Clarke (1917) and Gaskell and Millar (19_0) also describe phagocytosis of pigment by large cells lying in the lumen of the vessels which they regarded as desquamated endothelial cells Margulis states that the endothelial cells contain pigment Other careful observers have found little evidence of real phagocytosis by the true endothelial lining cells Arieti (1946) found that in two heavily parasitized cases of *P falciparum* infection phagocytosis of the endothelial cells lining the vessels was practically absent Parasites were sometimes seen attached to the vascular endothelium but were apparently never ingested Rigdon (1944) says phagocytosis by these cells is infrequent even in cases in which extreme phagocytosis is present in other organs such as the liver Taliaferro and Mulligan (1937) have examined the whole question of phagocytosis by various kinds of cells in the brain in malaria and have come to the conclusion that although phagocytosis of parasites and pigment may occur as the result of abnormal activity on the part of certain cells lining the vessels as a rule such activity is uncommon except in unusually severe cases and where stasis in capillaries is evident The phagocytosis which occurs in the brain under any conditions is inconsiderable compared with that going on in other organs even in the same patient In bird and monkey malaria Taliaferro and Mulligan found no localization of parasites in the brain such as may occur in *P falciparum* infections and no undoubted endothelial cells lining the capillaries contained pigment or parasitic material Pigmented cells were found in the lumina of the vessels similar to those sometimes seen in the human They consider the latter were not derived from endothelium but were haematogenous macrophages or cells that had migrated

Anatomical changes in the blood vessels

In acute severe malaria the vessels of the meninges and the brain substance are congested Where haemolysis has been intense e g in blackwater fever there may be an anaemia of the brain tissue with many small vessels apparently empty of blood In such cases however regional patchy congestion is common with the vessels filled tightly with erythrocytes

The walls of the smaller arterioles and veins and those of the capillaries commonly show some sign of damage The endothelium is frequently swollen and degenerate The affected cells may project into the lumen and according to many authors may ultimately become detached from the wall and lie free in the circulation Such detached cells are degenerate and may contain masses of pigment and parasitic debris (Marinesco 1921 Thomson and Annecke 1926 Dudgeon and Clarke 1917 Gaskell and Millar 1920) The possible phagocytic activity of the endothelial lining cells of the vessels has already been discussed Taliaferro and Mulligan (1937) believe that such cells are rarely phagocytic and that the so-called detached endothelial cells are in all probability macrophages from other parts of the body carried to the brain by the blood stream Arieti (1946) however has recently provided striking photographic evidence of endothelial hypertrophy and desquamation

The degenerative changes suffered by the endothelial cells have been recorded by various observers as simple granular degeneration (with or without vacuolation) fatty degeneration (with small or large globules of fat) or necrosis of both cytoplasm and nucleus (Marinesco 1921 Lafora 1912 Thayer 1899 Craig 1909 Torrioli 1932 Thomson 1924 Thomson and Annecke 1926 Gaskell and Millar 1920 de Vries 1927) All stages of degeneration may be met in the same brain Usually the degenerative changes are not severe but *Gaskell and Millar considered that they were one of the most significant* pathological effects of malaria in the brain both in regard to subsequent local neurological damage and haemorrhage into the brain tissues

New formation of blood vessels in the brain has been described by Corletti (1910) and Lafora and more recently by Arieti (1946)

The adventitial cells of the smaller arterioles and venules sometimes show signs of pathological changes including degeneration which may be severe and progressive (Lafora 1912) Lafora has stated that the adventitial cells may sometimes separate off from the vessels and penetrate *into the* brain tissue as the so-called *stabchenzellen* Arieti

found the adventitial cells of meningeal and cerebral vessels enlarged with strongly basophilic cytoplasm and nuclei rich in chromatin

Perivascular infiltration with cells has been described by some workers Gaskell and Millar for instance state that in their cases of cerebral malaria the perivascular lymph spaces were distended with lymphocytes and Marinesco observed lymphocytes plasma cells and monocytes in excess in the sheaths of the small meningeal vessels and infiltration of small vessels in the cerebral cortex and peduncles of the brain Margulis reported that in one case of *P falciparum* malaria of three months' duration there was no general infiltration of the vessels in the brain although in some areas in the white substance there was evidence of perivascular development of glial cells Other authors have found no indication of cellular reactions around the vessels Thus Dudgeon and Clarke describing lesions in cases clinically similar to those of Gaskell and Millar reported complete absence of any perivascular inflammation

In some cases of *P falciparum* malaria in which there was evidence of cerebral oedema the perivascular lymphatic spaces have been found distended Globules of fat have also been reported in these spaces

Vascular obstruction

Many authors refer to some form of blocking or obstruction of the capillaries and small arterioles and venules of the brain in malaria For instance Thomson and Annecke (1926) state that the most obvious lesion is more or less complete blockage of the brain capillaries with parasitized red cells which owing to their tendency to clump together and stick to the endothelial cells at the periphery of the vessel cause oedema stasis thrombosis and punctiform haemorrhages Marchiafava and Bignami (1894) describe the capillaries as loaded with red blood cells parasitized and non parasitized Taliaferro and Mulligan (1937) in their summary of literature mention obstruction of the capillaries by accumulation of parasitized red cells pigment and phagocytes occurring most commonly in *P falciparum* infections but also reported in *P vivax* malaria (Billings and Post 1915) Marchiafava and Bignami also refer to pigment obstructing the smaller vessels of the brain and state that in addition the vessels may be blocked by white coagula Thrombosis of the capillaries has also been described by Thayer (1899) and Dudgeon and Clarke (1917) but Gaskell and Millar in their cases of malignant tertian malaria found no conclusive evidence of thrombosis in the brain vessels In the apparently obstructed vessels the latter authors found the erythrocytes normal in shape and

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changes present in 100 cases of malignant tertian malaria These authors point out that text book illustrations of so-called plugging of cerebral vessels merely show capillaries filled with red cells mostly parasitized Plugging was not a major factor in any of their cases and there was little correlation between such packing of the capillaries and the clinical signs and symptoms manifested during life Thus of 56 patients classified as to the cause of death 12 had had typical cerebral malaria and 24 one or more major cerebral symptoms such as convulsions In 10 of the cerebral malaria cases there was no plugging in the other two there were parasitized cells in the brain capillaries but no obvious obstruction In 16 other cases none of which were classified as cerebral there was some evidence of plugging of the capillaries

As mentioned above similar results were reported by Gaskell and Millar (1920) in cases of malignant tertian malaria in Salonika In neither frank cerebral cases nor septicaemic infections in which there was extensive parasitaemia were these workers able to find any evidence to support the view that thrombosis of capillaries was present They considered that the circulatory disturbances in cerebral malaria were not primarily obstructive but were rather haemorrhagic in type Many capillaries were filled with intensely parasitized red cells and sometimes contained extracorpuscular parasites and free pigment but there was no evidence of the presence of fibrin Gaskell and Millar stated that their results indicated that circulation through such vessels was not necessarily wholly obstructed and that the assumption that thrombosis had occurred was unwarranted and unnecessary for the explanation of the pathological changes seen

Spitz (1946) has recently reported occasional thrombi in *P falciparum* infections in the central vessels of granulomas and in capillaries in the brain substance The thrombi were small and usually distended a small segment of the otherwise plugged capillary or arteriole In some vessels the thrombi only partly occluded the lumen From her study of 50 cases of malignant tertian malaria Spitz concluded in agreement with Rigdon that the small vessels of the brain were not obstructed by parasitized erythrocytes but simply filled and distended by them

Haemorrhages

Small perivascular haemorrhages suggestive of localized diapedesis of erythrocytes have often been seen in the brain in malaria particularly where the degree of general vascular congestion is considerable (Arieti 1946 Rigdon 1944 Gaskell and Millar 1920) The vessel

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Margulis found the brain capillaries blocked with red cells parasitized and unparasitized and malarial pigment in one case in regions where parasites were few no red cells could be distinguished in the vessels the lumina being filled with what he describes as strukturlosen rotlichen homogenen Masse He considered these vessels to be in a condition of stasis Hyaline bodies or masses filling the vessels have also been described by Lafora in acute malaria Other authors have described pigmented bodies obstructing the capillaries and giving rise to parasitic thrombosis (Rigdon 1942) These masses and bodies filling the lumen of the vessels are presumably present as a result of stasis of the red cells

Lafora observed in addition many congested capillaries containing parasitized red cells and sometimes free pigment and parasites Large mononuclear cells resembling macrophages containing malarial pigment and degenerated parasites were very common in some vessels Endothelial cells from the vessel walls were free in the lumen Phagocytes have also been reported in the brain capillaries by Taliaferro and Mulligan (1937) and Marinesco (1921) and others Marinesco mentions also lymphocytes monocytes and plasma cells and describes some vessels containing only lymphocytes or lymphocytes and polymorphs

The degenerate or hypertrophied swollen endothelial lining cells of the vessels have been regarded by some workers as partly responsible for obstruction to blood flow either directly or by assisting in the formation of thrombi (Marchiafava and Bignami 1900 Thayer 1899 Craig 1909 Lafora 191- Torrioli 1932)

Thus obstruction to the flow through the brain capillaries has been variously considered to result from stasis thrombosis embolism *the presence of large numbers of phagocytes and the hypertrophy* and degeneration of the lining endothelial walls Rigdon (1944) has analysed the available evidence and concluded that thrombosis and embolism are not significant factors in the production of the lesions of the brain in malaria Stasis similar to that occurring in shock and changes in the endothelial cells of the vessel wall are of more importance Rigdon considers that the so-called capillary obstruction is in many cases more apparent than real and points out that there is often little relation between the clinical signs exhibited by the patient and the lesions seen at autopsy In this respect his views are supported by the findings of Kean and Smith (1944) who studied the pathological

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tion Bastianelli and Bignami have suggested that the distribution may be related to the differences in the rate of blood flow through the grey



Fig 18—H m h ge nt b n n m l c nc e rc mal Note th g ted ent lly pl d t n l d ccntr nes f b n u nd t ted ed ll Th n b en c f gl l r p nw (Art A h and Sp tz 1946)

and the white substance resulting from the more elaborate capillary network of the former Arieti (1946) believes that the distribution of the haemorrhages is primarily dependent on the vascular pattern of the brain circulation The areas in which he found the greatest concentration of haemorrhages all possess a relatively poor blood supply (Cobb 193_) He infers from this that occlusion of a vessel in these regions probably could not be compensated for by collateral circulation as easily as in the better vascularized areas and changes in the vessel walls might consequently result leading to diapedesis of red cells There is apparently little direct relation between the appearance of the haemorrhages and the state of parasitization of the cells within the vessels since it is a common finding that the vessels of the grey matter contain more heavily parasitized blood than those of the white (Gaskell and Millar 19_0)

Degenerative changes in brain cells

The nerve cells are frequently injured during the course of severe malarial infection Marchiafava and Bignami (1894) observed some chromatolysis especially in cases of cerebral malaria and Lafora (191_) described extensive degeneration of ganglion cells in two cases with

involved is usually intensely congested and the contained erythrocytes frequently in a condition of stasis There are often associated degenerative changes in the endothelium the existence of which probably permits the initial escape of the red cells through the vessel walls Such perivascular haemorrhages are found more commonly about the vessels of the white matter than about those of the grey According to Gaskell and Millar haemorrhages into the brain substance in septicaemic malignant tertian malaria (i e in cases in which there is a generalized parasitaemia not localized in any particular organ) are confined to this type of lesion and are found especially associated with arterioles and not capillaries

The more extensive haemorrhages into the brain substance commonly described in all forms of severe malaria probably originate as extensions of the perivascular type They are usually clearly associated with some vessel According to Gaskell and Millar they occur more in relation to the smaller arterioles and venules than capillaries The vessels involved are usually packed with heavily parasitized cells Many authors e g Marchiafava and Bignami have described these haemorrhages as occurring round thrombosed vessels but as has been explained above it is more likely that the erythrocytes within the vessels concerned are in a state of stasis Degenerative changes in the endothelial cells of the vessels involved are commonly present There is frequently a narrow zone of brain tissue degenerative or apparently normal between the vessel itself and the red cells lying free in the substance but sometimes the cells are extravasated directly into the perivascular tissue

The brain tissue invaded by the red cells of the haemorrhage has been described as torn up The extravasated red cells in the tissue are often practically free from parasites although the neighbouring vessels may be filled with parasitized erythrocytes Occasionally the extravasated cells may also be parasitized Under these circumstances the parasites may be in an earlier stage of development than those found in the vessel itself

The distribution of these larger haemorrhages is the same as that of the perivascular type They occur mainly in the white matter and according to Gaskell and Millar are related most commonly to the smaller arterioles rather than capillaries The explanation of their frequency in the white matter and relative absence from the grey is not known Various suggestions have been made including the proposition that the vessels of the white substance are more primitive than those of the grey but there is very little evidence to support such a conten-

neuroglial cells often clustered around capillaries or degenerate nerve cells has also been described Similar cells have been ob erved in large numbers lying in amongst degenerate medullated nerve fibres in the white substance (Gaskell and Millar in septicaemic cases of mal gnant tertian malaria) Degenerative change in the glial cells have also been described including cystic degeneration of astrocytes (Lafora 1912)

Proliferation of glial cells is however most commonly seen in association with a group of lesions which have become known collectively as malarial granulomata Margulis (1914) noted scattered areas of softening in the brain of a patient who had died from malaria after an illness lasting three months He found many essels containing blood in a state of stasis surrounded by a narrow zone of degenerate brain tissue Beyond th s zone and sometimes mixed with the degenerated tissue were large numbers of extravasated erythrocytes often badly staining In some areas there was massive perivascular proliferation of glial cells Marinesco (19-1) observed in his cases nodules of proliferating neuroglial cells scattered through the white matter particularly in the subcortical regions near the grey matter they were also present in the thalamus lenticular nucleus and caudate nucleus Occasionally these nodules were composed mainly of lymphocytes mononuclears and sometimes plasma cells but such accumulations of cells were usually surrounded by a peripheral collection of neuroglia

Similar cellular accumulations have been described by many other authors Thomson and Annecke (19-0) examined the brains of 1- cases of malignant tertian malaria and found nodular lesions in three in the cortex and in one in the cerebellum They were as Margulis had stated commonest in the subcortical regions of the white matter near the deep layers of the cortex and were closely associated with numerous punctiform haemorrhages The characteristic nodule was composed of a central congested capillary containing stased parasitized red cells a narrow perivascular area of necrotic or degenerative brain tissue and an outer circle of proliferating glial cells intermingled with extravasated red cells usually free from parasites The glial cells were often peripherally arranged giving the whole lesion a superficial resemblance to a tubercle The glial cells sometimes showed active mi osis

Thomson and Annecke found capillary haemorrhages in relation to all their nodules and considered that such haemorrhages were the precursors of the cellular reaction the latter arising as a tissue response

mental symptoms which died in coma The cells in these cases showed chromatolysis and the cytoplasm was filled with granules to the exclusion of the Nissl bodies The medullated nerve fibres were not affected Margulis (1914) also described degenerative changes in the nerve cells *in a case of malignant tertian malaria which died after a* three months illness with large liver and spleen and failing right heart. There were scattered areas of softening in the brain and in these regions the nerve cells showed degeneration of Nissl granules and peripherally placed nuclei Margulis concluded that the lesions were not specific to malaria but were adequate to explain the presenting clinical signs Dudgeon and Clarke (1917) also described extensive degenerative changes in nerve cells in three cases of *P falciparum* infection Gaskell and Millar (1920) made a careful study of the nervous lesions in both cerebral and septicaemic forms of malignant tertian malaria In the former they found varying degrees of injury in the nerve cells including irregular fatty degeneration The medullated fibres also showed degenerative changes In the latter the lesions were more marked The nerve cells were degenerate In some cells the nuclei were extruded in others they were excentrically placed and stained badly often showing no nucleolus The protoplasm exhibited granular degeneration and frequently contained a peppering of fat globules *The medullated nerve fibres were surrounded by round cells* and showed varying degrees of degeneration

Degeneration of nerve cells and fibres is sometimes general in distribution but more often occurs in localized scattered areas sometimes concentrated in one or more regions of the brain Durck (1925) for instance described degeneration particularly affecting the Purkinje cells and Rigdon and Fletcher (Rigdon 1944) observed extensive nerve cell degeneration especially in the Purkinje cells in man monkeys chicks and ducks

Neuroglial changes, 'granulomata'

Reference has already been made above to the proliferation of neuroglia in the neighbourhood of the brain capillaries especially in the white substance (Margulis) This phenomenon has also been recorded by other observers e g Lafora (1912) Arieti (1946) and Marinesco (1921) the latter stating that neuroglial cells undergo hypertrophy as well as multiplication A more general increase in glial cells has been noted in the white substance by Marinesco and Gaskell and Millar in cerebral cases of malignant tertian malaria Infiltration of the brain tissue with round cells which may possibly have been

reaction to a malarial toxin and thought the lesions were capable of producing permanent multiple sclerotic areas Seyfarth also considered such lesions might give rise to a multiple sclerotic condition He stated that the brain never completely recovered from an attack of malaria in which the lesions had advanced to the stage of formation of granulomata No scarring of the brain as a sequel to malaria seems however to have been described

PATHOGENESIS

The summary given above of the pathological changes found in the brain in acute malaria associated or not as the case may be with a cerebral clinical state shows clearly that the most consistent lesions are vascular involving the capillaries and small arterioles of the brain tissue especially in the white substance The affected vessels are dilated and congested or in a condition of stasis and are commonly filled with parasitized erythrocytes The endothelial lining cells of the vessels show degenerative changes and perivascular escape of erythrocytes leading to macroscopic haemorrhage occurs Associated with these vascular changes there are signs of degeneration and regeneration of the brain tissue including degeneration of the nerve cells and the formation of so-called granulomata and various other accumulations of glial and lymphocytic cells Rigdon (1944) separates the brain lesions into three groups (i) the vascular and haemorrhagic (ii) degenerative and (iii) cellular reactions Of these the first is undoubtedly of major importance

The general appearance of the vascular lesions has often been taken to indicate obstruction of the blood flow through the smaller vessels of the brain and for many years attempts have been made to demonstrate such impedance of flow and the mechanism of its production

Marchiafava and Bignami (1894) pointed out that the smaller vessels of the brain substance contained relatively larger numbers of parasitized cells than the larger vessels and tried to link this fact with the apparent obstruction to the circulation in the small vessels on the grounds that parasitized cells offer more resistance to flow through capillaries than normal cells Such increased resistance would lead to slowing of the blood flow and this in turn would result in interference with the metabolism of tissue including the endothelial cells so accounting for the degenerative changes found in the latter Diapedesis of red cells through the changed vessel walls would follow and give rise to the perivascular leakage of blood and ultimately to full scale haemorrhage

to injury and going on in the event of recovery to repair They believed that scarring would ultimately result

Weingartner (1920) described similar lesions and found them always related to a vessel He considered they arose from glial proliferation about an established extracapillary haemorrhage Marinesco also noted that they were always associated with haemorrhages and placed along the course of small blood vessels Durck on the other hand found that although usually related to blood vessels the nodules sometimes occurred independently in the brain substance He considered they were comparable to true granulomata and should be regarded as defensive inflammatory processes In his series of patients nodules of this sort were found in all cases of pernicious malaria. Arieti however does not regard these nodules as inflammatory phenomena and suggests the term pseudogranuloma should be used to describe them This view is probably correct since very similar lesions occur in fat embolism (Spitz 1946)

Nodular lesions have been reported occasionally in other forms of malaria Oesterlin for instance has described them in a case of benign tertian and in one case of blackwater fever Freifield has observed them in quartan malaria

Margulis attributed the formation of these nodular lesions to the effects of vascular stasis and suggested that the glial proliferation represented an attempt at repair Marinesco believed they resulted from the irritative action of the local haemorrhage or the production of a malarial toxin Durck also suggested they arose from the tissue

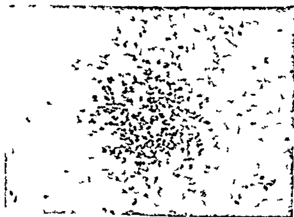


Fig 19—Malarial granuloma in the subcortical white matter of a case of malignant tertian malaria

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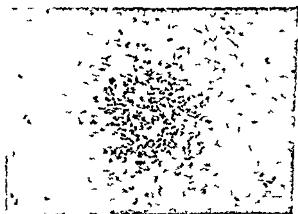


Fig 19—Malarial granuloma in the subcortical white matter of a case of malignant tertian malaria

circulating fluid and consequent concentration of erythrocytes i e stasis To some extent both these processes appear to be involved

Knisely (1945) describes a thick glassy precipitate forming between and around the erythrocytes in the late stages of acute malaria in man monkeys and birds developing throughout the circulation contemporaneously The erythrocytes are bound together in small wads and masses resembling sludge which offers resistance to its own progress through the small vessels Such resistance slows the circulation and leads to a state of progressive stagnant anoxia The anoxic conditions bring about changes in the vascular walls and leakage of plasma fluid takes place into the surrounding tissue In the vessels most affected stasis of erythrocytes occurs around the sludged cells and obstruction to blood flow may become complete There is little doubt that the phenomenon of sludging so clearly demonstrated by Knisely in the general circulation in malaria plays some part in the vascular changes in the brain It is difficult to separate it from stasis developing from other causes particularly as in each case the appearance of the obstructed vessels would be practically identical the individual corpuscles becoming welded into a homogeneous mass (Florey 1926 Marinesco 1921 Margulis 1914) Sludged blood should however show evidence of the presence of fibrin and as has been pointed out above fibrin is not often found in the capillaries in malaria (This fact is also of importance in considering the possibility of the existence of other forms of increased agglutinability of the red cells)

It is possible that the cerebral circulation is more sensitive to changes in the erythrocytes than the circulation elsewhere and that vascular phenomena commence there at an early stage before any degree of sludge agglutination of red cells has occurred Knisely found that the erythrocytes in the final stages of *P knowlesi* malaria in monkeys were sticky in relation to one another and to phagocytes but were not apparently so to the endothelial lining of the blood vessels Many other authors have however described the appearance of stickiness particularly of parasitized cells in human malaria and have suggested that the slowing of the circulation might be effected to some extent by this phenomenon The great accumulation of parasitized cells in the smallest vessels and their frequent distribution along the endothelial walls of the larger vessels are often quoted as evidence of such stickiness Another factor which may sometimes play an important part in impeding blood flow in the small vessels is the appearance of spherocytosis (Whipple 1941)

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These authors also noted that the extravasated cells in the punctiform haemorrhages were not usually parasitized although those in the associated vessel were heavily infected and suggested that this indicated that some degree of stagnation had occurred in the vessel They considered that the degenerative changes in the nervous tissue resulted from the interference with circulation

The view that the presence of parasitized cells may be a primary factor in bringing about slowing of the cerebral circulation has also been advanced by Bass and Johns (1915) These authors believed that the plasmodial consistency was greater than that of the red cells Consequently the presence of intracorpuscular parasites led to greater difficulty in transit of erythrocytes through small bore vessels such as capillaries and thus to interference with blood flow and finally obstruction of the vessels concerned

Marchiafava and Bignami believed that true thrombosis also occurred in the brain capillaries and played a significant part in the interference with blood flow Many other authors have expressed similar views but as has already been pointed out there is little firm evidence to indicate the existence of any marked degree of thrombosis or embolism in the capillaries of the brain in malaria Rigdon (1944) as mentioned above has reviewed the literature on this point and concluded that both emboli and thromboses must be extremely uncommon in malaria and that obstruction to the vessels if it occurs probably results from stasis of the erythrocytes and not from thrombosis The frequently rapid response of patients with cerebral malaria to specific drug therapy indicates as Dudgeon and Clarke (1917) have pointed out that many of the lesions in the organs including those in the brain can only be of a temporary or reversible nature Blocking of vessels cannot therefore be entirely due to thrombosis or embolism since these phenomena would be uninfluenced by specific drug therapy

If the interference to the cerebral blood flow is not caused by thrombosis or embolism it may in part be due to purely mechanical phenomena resulting from physical changes in the invaded erythrocytes as suggested by Marchiafava and Bignami and Bass This can apply however only to the passage of heavily parasitized blood through the small vessels and cannot explain the impeded circulation in areas where the degree of parasitization is not extensive It is clear therefore that other mechanisms which are rapidly reversible must be at work tending either to cause the corpuscles to cling together and to the walls of the vessels or to give rise to changes in flow resulting from loss of

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erythrocytes and the development of spherocytosis are possibly only some of the phenomena which work in the one direction of slowing the circulation rate through the small vessels The common result of all these processes is some degree of interference with the well-being of the endothelial lining walls of the vessel the effects of which are often visible as degenerative changes The damage to the vessel wall results in alteration in its permeability Fluid passes in abnormal amounts through the cells into the surrounding tissue and is removed at first by the lymphatic drainage Eventually the fluid loss is sufficient to alter the local circulatory plasma volume, and as the intravascular fluid steadily decreases stasis of the red cells develops The individual red corpuscles tend to become merged into an apparently solid homogeneous mass and circulation through the affected vessel ceases In areas where the process is less advanced the flow may be greatly reduced but not completely stopped and the aggregation of corpuscles does not take place The red cells will under such conditions still appear separate In most vessels this is the usual pathological finding Gaskell and Millar have pointed this out as evidence against the existence of thrombosis and have stated that the circulation in such vessels is incompletely obstructed They consider the assumption of the existence of thrombosis unnecessary to explain the presenting pathological changes

Gaskell and Millar held that the chief circulatory disturbances in the brain in malaria were the haemorrhages which occurred about the vessels rather than the changes in flow through the vessels themselves In so far as the more serious and long-lasting nervous lesions of malaria are concerned this view is probably correct but the lack of correlation between such serious tissue damage and the severity of the clinical signs and symptoms of nervous involvement makes it clear that processes other than haemorrhage must play a vital part and it is difficult to see how such processes can be related to anything else than temporary changes of flow through the brain blood vessels Such changes in flow must be due to reversible phenomena since they can be so quickly and thoroughly restored and it appears that stasis or near-stasis of erythrocytes resulting from temporary loss of circulating plasma fluid, must be the main factor at work.

The development of obstruction or impedance to the flow through the small vessels as a result of processes leading to stasis is thus probably the most significant phenomenon in the pathogenesis of the brain changes and clinical features of the cerebral syndrome in malaria The mechanisms by which such circulatory changes can be brought

about are of fundamental importance to the understanding of the condition

The basic disturbance giving rise to stasis is one by which the fluid escapes into the surrounding tissues from the plasma circulating through the vessel This escape of fluid is brought about by changes in the vessel wall which render it permeable to substances to which it is normally impermeable Protein and fluid resembling plasma may thus pass through the endothelial lining and where the circulation is slow the loss of fluid may take place so rapidly that a local reduction in circulating fluid volume occurs The erythrocytes are concentrated as a result of the fluid loss and finally become compressed together into what Florey describes as a transparent mass of corpuscles When this stage is reached flow through the vessel ceases and all the fluid appears to be drained away from the lumen Restoration of flow commences by the gradual escape of individual corpuscles from either end of the stased column of erythrocytes into the circulation (Florey 1926) It is thus clear that there is no true agglutination of corpuscles during stasis and the condition appears to be fundamentally a reversible one With the resolution of stasis fluid returns to the lumen of the vessel and the impermeability of the lining cells is restored unless some kind of permanent damage to the endothelium has taken place

Stasis of the cells within a vessel leads to interference with the oxygen supply to the tissues surrounding it and if this state of affairs continues for any length of time damage to the tissue will result from the effects of anoxia and the accumulation of metabolic products

The lesions of the brain in malaria can thus be adequately explained on the grounds of stasis and subsequent tissue damage resulting partly from interference with circulatory flow and partly from the effects of extravasated red cells passing between and through the endothelial cells of the affected vessels The problem appears to be fundamentally a matter of discovering what processes are at work in producing the changes in the vessel walls which lead to the escape of fluid and red cells into the tissues There seems to be little doubt that the primary derangement is one of anoxia (Landis 1927 Florey 1926 Moon 1938)

If we are to postulate the existence of a state of anoxia in the brain in malaria we must examine the means by which it can be produced Anoxia can develop as the result either of local or general factors arising partly from the direct effects of the parasite on the red cell and partly from the reaction of the body to the infection

Local anoxia may arise in the first instance as in other parts of the body as the result of the invasion of the erythrocyte by the parasite

The oxygen requirements of the parasite are such that in a heavy infection the demand is sufficient to make an appreciable difference to the oxygen available to the tissues (see Chapter II) It has been explained elsewhere that the parasite probably normally takes its oxygen from the oxyhaemoglobin of the invaded cell and converts *some of the haemoglobin into inert malarial pigment rendering the* invaded cell inefficient as an oxygen carrier to the tissues It is also possible that the unparasitized red cells may be affected and that the formation and dissociation of oxyhaemoglobin may be disturbed The loss of both parasitized and unparasitized cells by lysis may also greatly reduce the available oxygen supply and make the tissues more than usually susceptible to changes in the circulation of the blood It might be argued that in severe malaria the reduction in circulating blood volume sometimes seen may be sufficient to compensate for the loss in red cells but measurement of haematocrit values in severe *P falciparum* infections has shown that this is not the case (Feldman and Murphy 1945)

General anoxia arising from failure of the circulation must also be considered an important factor The action of malaria on the heart muscle may also lead on occasion to cardiac failure with characteristic changes in the circulation in the brain (Gaskell and Millar 19-0) In severe acute malaria or blackwater fever vascular failure closely allied *to medical shock also affects the cerebral circulation (Rigdon 194-* Maegraith 1944 Kean and Taylor 1946)

These general considerations are referred to elsewhere It is necessary here merely to point out that conditions exist in the brain in malaria which are capable of giving rise to anoxia of one form or another in the brain tissue including the walls of the blood vessels In the experimental work of Florey and Landis and others in which the effect of anoxia on the permeability of capillaries was examined the anoxia was acute and the effect reversible Anoxia acting over a long period has more permanent effects on the vascular endothelium ultimately giving rise to degenerative changes and finally necrosis Changes of this sort are often seen in the endothelial cells of the vessels in malaria (see above) and Cannon (1941) has suggested that they may in themselves predispose to leakage of fluid from the lumen into the perivascular tissues He says One feature of pernicious malaria which is noteworthy is the vascular injury as revealed by generalized fatty degeneration Such a condition should certainly predispose to loss of fluid elements of the blood similar to that in shock and as it does particularly in the algid forms of pernicious malaria He also suggests

that the haemoconcentration of pernicious malaria may assist the tendency to blockage of the capillaries but as has been pointed out above this is unlikely if there is associated severe haemolysis

It will be seen that the lesions in the brain in malaria can be reasonably attributed to the production of stasis or near-stasis of the erythrocytes in the small vessels giving rise to interference with blood flow and changes in the vascular walls allowing diapedesis of red cells or more severe haemorrhage The changes in the vessels and the blood flow through them have been attributed to anoxia arising from a number of causes some local some more general

It remains to discuss the creation of anoxic conditions more fully

The lesions described are not specific to the parasitic invasion Similar changes may arise as the result of induced hyperthermia severe anaemia mechanical interference with blood flow to the brain or the use of certain narcotic drugs including barbiturates and alcohol Hartman (1937 1938) compared the lesions of cerebral malaria with those produced in man and dogs by the artificial production of fever ranging from 104 to 109 F He found that high fever gave rise to degenerative changes and haemorrhage in the adrenals liver brain and other organs which were comparable with changes in these organs produced by heat stroke burns and anoxia caused by ligation of blood vessels or the use of lethal doses of nitrous oxide gas The pathological changes produced in fever therapy were related to the height of the fever and its duration At blood temperatures ranging from 104 to 107 F the oxygen saturation of the femoral arterial blood was reduced if the fever were continued for four to five hours In animals this saturation fell steadily and was associated with lethal changes beyond a certain percentage (65 per cent saturation) The severity of the lesions was increased as the oxygen saturation of the blood was depressed

The first signs of changes in the tissue were congestion followed by oedema indicating increased permeability of the vessel walls This was frequently succeeded by cuffs of haemorrhage about the minute vessels and changes in the cortical basal ganglionic and cerebellar cells which stained poorly and sometimes contained pyknotic nuclei At a later stage so-called devastation areas (Gildea and Cobb 1930) were found in both human and dog brains There were areas of necrosis of varying size containing poorly staining cells with pyknotic nuclei interspersed with clear zones of loose tissue These areas were similar in some respects to the scattered areas of necrosis seen in the brain

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such as occurs in malaria and which can be artificially induced by breathing an oxygen-deficient atmosphere the reduction oxygen in tension in the circulating blood results in a deficiency of total oxygen supply to the brain tissue Lesions develop in the central nervous system especially in the cortex and basal ganglia involving both vessels and tissue cells These changes are at first physiological and reversible but after a short time become irreversible and result in permanent damage (van Liere 194- Wolff 1936 Schmidt 19-8 1935 Schmidt and Pierson 1934) In conditions of anaemic anoxia which also occurs to some extent in malaria and which can be produced experimentally by the occlusion of the arterial blood supply or by breathing carbon monoxide non specific brain lesions develop which include haemorrhages about the vessels and circumscribed areas of necrosis involving both nerve and glial cells In such circumstances the cells of the cortex are affected first and become permanently damaged in the shortest time so that as Drinker points out the animal subjected to severe anoxia becomes virtually decerebrate (Gomez and Pike 1909 Gildea and Cobb 1030 Weinberger *et al* 1940) It will be seen that the lesions described closely resemble those occurring in malaria

Many attempts have been made to discover whether the malarial plasmodia produce toxins capable of causing the tissue lesions seen in the disease Such work has not met with much success and at the moment it is probably fair to say that no such toxins have ever been demonstrated This may be because they have been sought in the wrong place Certain authors have however from time to time tried to implicate the products of schizogony in the pathogenesis of malaria particularly the release into the circulation of the plasmodial pigment haemozoin now believed to be haematin This hypothesis has recently been exhaustively examined by Anderson Morrison and Williams (1942) and Anderson and Morrison (194-) These authors injected disodium ferri haemate in large doses intraperitoneally intravenously and subcutaneously into dogs and analysed the results They found that when injected in large doses intraperitoneally or intravenously the pigment caused serious lesions chiefly involving the blood vessels There was generalized vasodilatation and extravascular haemorrhage thrombosis and infarction also occurred The subarachnoid space was congested and peppered with petechial haemorrhages Most of the lesions produced could be accounted for by multiple small thrombi The pigment was harmless when contained in phagocytes but poisonous when injected into the blood stream and the lesions were secondary to circulatory or vascular changes rather than to any direct toxic

in malaria but lacked the obvious cellular reactions of the malarial lesions

Hartman attributed these lesions in hyperthermia to anoxia He pointed out that anoxia could arise in four ways (Peters and van Slyke 1931) namely as anoxic anaemic stagnant or histotoxic anoxia and discussed the various possibilities of the development of such states He considered that the factors concerned in producing anoxia in hyperthermia were alkalosis the *increased temperature* of the blood (resulting in decreased oxygen saturation Barcroft 1920) increased blood flow through the brain (at first) and the increased tissue demand for oxygen (resulting from increased metabolism and depression of oxygen-utilization by the tissues)

The alkalosis arose from the rapid breathing developed in hyperthermia which led to a lowering of plasma carbon dioxide and a measurable shift of blood pH to the alkaline side (Bischoff Long and Hill 1931) It had the effect of inhibiting to some extent the dissociation of oxyhaemoglobin The effect of high blood temperatures on the oxygen saturation of arterial blood has already been mentioned This was at first associated with an increased blood flow through the tissues the heat giving rise to a decrease in circulation time but as the fever progressed the cardiac function began to fail and stagnation of the circulation and further reduction in oxygenation of the blood and tissues resulted The damage to the smaller vessels was probably *initiated by increase in the permeability of their walls to* fluids and proteins Moon (1938) supported this view since he found the changes in the vessels in hyperthermia were identical with those seen in shock and Kopp and Solomon (1937) reported clinical shock in patients in whom hyperpyrexia had been induced by means of hot moist air

Hartman concluded that the lesions of cerebral malaria were so similar to those of hyperthermia and other conditions arising from anoxia of one form or another that it was reasonable to deduce that the damage in malaria arose from the same basic pathogenic factor Before continuing this argument it is necessary to review the evidence with regard to the effect of induced anoxia on the central nervous *system and to consider other alternatives e g that the malarial lesions* arise directly from the effects of invasion or indirectly from substances such as haemozoin formed by the plasmodia

The effects of pure anoxia on the central nervous system have been recently reviewed by van Liere (1942) Oxygen deficiency first produces vasodilatation and an increased flow of blood through the brain In spite of this increase in flow however in anoxic anoxia

the two bloods and concluded that more glucose was removed from the blood by the brain tissue than by other tissues Measurements of the respiratory quotient of brain tissue showed it to be approximately 1 0

It is thus evident that brain tissue metabolizes glucose almost exclusively and must therefore depend fundamentally for its function on an adequate supply of both oxygen and glucose Reduction in either oxygen supply or glucose supply or both would cause serious derangements in the function of the nerve cells It has been mentioned above that anoxia can give rise to electrical silence—the equivalent of metabolic change in the nerve cells—and it has been found that similar silence can be brought about by prolonged hypoglycaemia and restored by the administration of glucose (Hoagland 1937) Quastel (1939) quoting experiments performed by Himwich and Fazekas concluded that suppression of nerve cell activity (produced by suppression of oxidative systems) could be brought about by hypoglycaemia even in the presence of adequate oxygen

Stone and his colleagues (1941) investigated the metabolism of glucose during cerebral anoxia in anaesthetized cats and found that in anoxia produced by either ligating one carotid artery or breathing an oxygen deficient atmosphere lactic acid accumulated inorganic phosphate increased and the concentration of phosphocreatine diminished The processes were reversed if the animal were allowed to recover The changes observed in phosphorus and phosphocreatine content of the brain tissue indicated interference with the breakdown and resynthesis of the latter The rapid disappearance of lactic acid on recovery was due to the activity of reversible lactic acid dehydrogenases and not to diffusion from the tissues to the blood since the rate of diffusion of lactic acid was found to be very slow

It has been shown by other workers that the electrical activity of the cerebral cortex depends on the maintenance of an adequate supply of oxygen and glucose Stone and his colleagues found that electrical silence developed in anoxia shortly after the decrease in phosphocreatine concentration and before there was any appreciable change in adenylpyrophosphate They interpreted this finding as indicating that the electrical energy is not derived from the breakdown of phosphocreatine but from oxidation of glucose by some alternative route not dependent on the phosphorylation cycle It is not surprising therefore that a deficiency of glucose supply to the brain complicates the effects of anoxia Hypoglycaemia which occurs during the malarial paroxysm and in the later stages of fatal malaria (Marvin and Rigdon 1945) has been found to aggravate the cerebral reactions to anoxia (Sugar

effect They concluded that the tissue damage arose primarily from anoxaemia following vascular obstruction and that it was not possible to implicate the parasite pigment as a specific toxic factor since it was never liberated in soluble form from the parasites

The evidence available at present thus indicates that the lesions of the brain seen in malaria can be most easily explained on a basis of prevailing anoxia in that organ produced primarily by changes in blood flow The gross pathological changes manifested in the central nervous system as a result of such exposure to anoxia are almost certainly reflected in derangements of the metabolism of all the cells of the tissue concerned This is especially so in the case of the nerve cells which are excessively sensitive to changes in oxygen concentration in their environment Exposure to carbon monoxide asphyxia (anaemic anoxia) for instance brings about irreversible changes in the small pyramidal cells of the cerebrum within eight minutes and in the cells of the medulla oblongata in 20 minutes (Drinker 1938) Weinberger (1940) produced permanent and severe changes in the cerebral cortex in even shorter time as a result of exposure to anoxic anoxia following occlusion of the pulmonary artery

The processes giving rise to such damage in the nerve cells have not been fully determined but it is evident that the local environment of the cells must alter considerably in any anoxic state Stone Marshall and Nims (1941) for example have shown that anoxia produced by breathing air containing low oxygen concentrations gave rise to a decided shift of blood pH to the alkaline side whereas in anoxic anoxia arising from occlusion *of the blood supply to the brain the pH* was reduced Accumulation of lactic acid occurred in conditions of abnormal alkalinity or acidity (depending upon whether the plasma carbon dioxide could be removed) and in the presence of metabolites of incomplete tissue oxidation The reaction to such environmental conditions was shown by the development of electrical silence *during the period of anoxia*

Under conditions of anoxia therefore changes in the metabolism of the brain cells are to be expected and should be observed particularly in regard to the forms of metabolism most sensitive to oxygen lack i e in this case the metabolism of carbohydrates

Lennox (1936) showed that the oxygen content of the blood in the internal jugular vein (representing blood coming from the brain) was much lower than that in the external jugular vein (representing blood coming from other tissue) The same worker (1931) and Myerson and Halloran (1930) found similar differences in carbohydrate content in

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the use of nicotinic acid in malaria is its vasodilator effect on the cerebral vessels an effect which would tend to minimize stagnant anoxic conditions

The above will illustrate the far-reaching effects of anoxia *per se* on the anatomical structure and physiological activity of brain tissue Whether in malaria these effects are aggravated and stressed by more specific activities on the part of the parasite or its products is yet to be determined A great deal of experimental work needs to be done before we can obtain the answer In the meantime the most reasonable hypothesis is the one we have outlined above namely that the changes produced in the central nervous system in malaria are basically determined by the creation of anoxic states

Rigdon (1944) has stated this proposition very clearly He points out that the cerebral lesions in malaria are not specific to the disease Identical changes occur in other conditions such as hyperthermia in which anoxia is the principal pathogenic factor The lesions in the brain in malaria thus most probably arise from the effects of anoxia. Anoxia may arise in malaria from the effects of parasitization of red cells and subsequent interference with oxygen carriage It also results from circulatory disturbances of which there is ample evidence in malaria both clinically and pathologically arising from cardiac failure or circulatory collapse The lesions in the brain tissue result first from increase in permeability of the walls of the small vessels brought about by anoxia giving rise to escape of fluid into the tissues The escape of fluid leads to slowing of the intravascular flow and ultimately in some vessels to stasis Associated anoxia of the parenchyma develops and may lead to metabolic changes and later to focal degeneration and ultimately necrosis When the damage to the capillary walls is sufficiently severe erythrocytes will escape by diapedesis and give rise first to perivascular haemorrhage and later to more extensive bleeding into the tissues The picture is complicated by the invasion of erythrocytes by the plasmodia since the parasitized cells appear to localize along the endothelial walls of the vessels and may interfere still further with the function Unless the anoxic conditions are rapidly relieved the metabolic activity of the tissue cells will become progressively impaired at first reversibly and finally irreversibly giving rise to permanent damage

and Gerard 1938) and Gellhorn (1940) believes that it acts synergistically with anoxia in the production of convulsions in animals exposed to anoxia Dameshek and Myerson (1935) found that during hypoglycaemia induced by insulin there was considerable reduction in glucose utilization and oxygen consumption by brain tissue In dogs there is an accompanying transient increase in blood flow in the cerebral branch of the jugular vein followed by a marked decrease in flow in both arterial and venous vessels There is thus a general reduction in blood flow through the brain so that as Quastel points out (1939) lack of glucose will create in the brain a state equivalent to anoxaemia even if oxygen is present for the latter element cannot be utilized

In the development of central nervous system derangements in malaria the general processes of anoxia may thus be modified by the presence of hypoglycaemia especially in the later stages of the disease Other factors are probably also involved For instance Sugar and Gerard (1938) have suggested that increase in extracellular potassium concentration (which occurs in malaria) may influence cerebral metabolism The complicated nature of the processes concerned in the oxidation of carbohydrate allows for many possible points of failure Apart from its action in the phosphorylation cycle which has been referred to above lack of oxygen leads to the failure of decarboxylation of pyruvic acid and the production of lactic acid as Stone *et al* have shown The utilization of oxygen in this case depends on the activity of the cytochrome oxidase system and the coenzymes the pyridine nucleotides flavoproteins and thiamine pyrophosphate which contain members of the vitamin B complex

As has been shown above stabilization of oxycytochrome is effected by histotoxic anoxia and may play an important part in impedance of metabolism in the brain in malaria Failure of synthesis or activity of the coenzymes may also be concerned but so far there is no concrete evidence of this Trupp (1946) has suggested that massive destruction of available nicotinic acid occurs in malaria during the phase of parasite multiplication If this were the case the synthesis of the pyridine nucleotides might be disturbed and the carbohydrate oxidation processes correspondingly depressed Trupp believes that the basis of nicotinic acid therapy in cerebral malaria is the restoration of the vitamin lost during the plasmodial development and that there may be competitive activity between the parasite and host for substances such as respiratory pigments and coenzymes No information on this point appears to be available The commonly accepted rationale for

necessarily regularly but often in a series of bursts of enlargement and apparent regression The spleen sometimes varies in size with the clinical state of the patient For instance many authors have noted that it enlarges during the paroxysm becoming smaller again in the apyrexial intervals

In long sustained malaria or under conditions in which subjects are exposed to frequent reinfections the spleen may become enormous Its ultimate size is related to the age of the individual and the number of previous malarial attacks The degree of enlargement resulting from repeated infections is usually greater than that following a long continued single infection Such enlargement is often seen in individuals displaying no obvious clinical signs of overt malaria and is probably related to the development of acquired immunity to the malaria parasite (Taliaferro and Mulligan 1937 Boyd 1930 etc) Napier (1946) regards splenic enlargement of this type as evidence imperfect host parasite adjustment No detailed account of this relationship between splenic enlargement and the development of immunity in human malaria is called for here The reader is referred to the many excellent accounts of the subject (Culbertson 1941 Coggeshall 1943 Wilson 1939 Thomson 1933)

Occasionally in chronic malaria fibrotic changes in the spleen may bring about a reduction in its size sometimes to smaller than normal Reduction has also in rare instances been observed in acute malaria (Scheube 1903)

In blackwater fever the spleen is usually palpable and tender The size depends to a large extent upon the malarial history of the patient

The enlarged spleen of acute malaria is often very tender and accompanied by considerable pain arising from stretching of the splenic peritoneum or according to some authors (e g Bispham 1944) to the development of perisplenitis The edges are soft and rounded and often ill defined on palpation In chronic malarial infections or after repeated infections the organ can often be palpated three or more fingers breadth below the costal margin and may be enormous reaching almost to the pelvic brim In such cases it is firm and presents well-defined sharp edges The ligaments supporting the spleen may stretch and allow the organ to move about more freely in the abdomen giving rise to the clinical picture of so-called floating spleen The enlarged organ is peculiarly susceptible to injury and may rupture as a result of external violence or more rarely spontaneously (Hughes and Niesche 1945 Andrew 1945) Infarcts twisted pedicle gangrene and even abscesses arising from secondary infections

CHAPTER X

THE SPLEEN AND BONE MARROW

THE SPLEEN Clinical Pathological Changes Macroscopic appearances — Histological appearances Parasites Pigment Vascular system Degeneration Phagocytosis and hyperplasia Phagocytosis in simian malaria Pathogenesis Circulatory changes — Degenerative changes — Phagocytosis — Hyperplasia

THE BONE MARROW Clinical Changes in blood and marrow Pathological Changes Macroscopic appearances — Histological appearances Vessels and parasites Haemopoietic tissue Leucopoiesis Phagocytosis Hyperplasia Pathogenesis Bone marrow response to anaemia — Anoxia

THE SPLEEN

In overt malaria the spleen is almost always enlarged and is often palpable (i e enlarged to about three times normal size or more) even in a primary attack after the first few days of the illness In *P vivax* infections for instance the spleen is usually palpable seven days or more after the first appearance of the parasites in the peripheral blood (Stratman-Thomas 1941) The size attained and the rapidity of enlargement depend on the infecting Plasmodium the age of the patient and the history of previous exposure to malaria The degree of enlargement other things being equal is roughly proportional to the duration of the illness (Boyd 1941) and is proportionately greater in children than in adults Specific treatment or spontaneous cure of an infection are followed in most cases by a steady reduction in splenic size Relapses or recrudescences are likely in cases in which the spleen does not subside after treatment of the primary attack Craig (1909) found that in naturally acquired *P falciparum* infections the spleen was more frequently enlarged (palpable) than in either *P vivax* or *P malariae* infections Kitchen (1941) reported opposite findings with regard to induced *P vivax* and *P falciparum* infections In the recent war enlargement of the spleen was very commonly observed in fresh *P falciparum* infections e g in British troops in West Africa Most authors agree that in *P malariae* infections enlargement of the spleen occurs late and is not usually as prominent a feature of the disease as in other infections Kitchen states that in *P malariae* infections the period of detectable enlargement is proportional to the duration of the clinical attack In all untreated infections the size of the spleen tends to increase as the illness progresses not

necessarily regularly but often in a series of bursts of enlargement and apparent regression The spleen sometimes varies in size with the clinical state of the patient For instance many authors have noted that it enlarges during the paroxysm becoming smaller again in the apyrexial intervals

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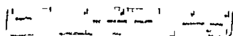


FIG 20—Enormous splenic enlargement in a case of recurrent malignant tertian malaria

Enlargement of the spleen in acute infections is a frequent finding in the malaria of both birds and monkeys Taliaferro and Mulligan (1937) state that in general the findings in these animals are similar to those reported in human malaria with comparable histories In monkeys the increase in size of the spleen depends upon the species of invading Plasmodium and the length of the infection Coggeshall (1937) has studied the variations in size of the partially exteriorized spleen in experimental *P knowlesi* and *P inui* infections of *M mulatta* Animals given a large intravenous dose of *P knowlesi* died in about five days after infection and showed an average increase in size of spleen of about 57 per cent above normal After a smaller dose of *P knowlesi* animals survived for about 12 days and showed an increase in splenic size of 91 per cent above normal The rate of increase in the size of the spleen was progressive until within 24 hours of death at which point it remained stationary or declined When the *P knowlesi* infection was made chronic by administration of quinine or atebrin there

was a much greater degree of splenomegaly Superinfection of these animals with homologous parasites resulted in a very rapid and considerable increase in splenic size followed by gradual return to the size before superinfection In monkeys inoculated with *P inui* the spleen increased in size during the infection and after reaching a maximum size remained constant for the period of observation In the first week the organ was soft and red suggesting congestion it then became firmer Afridi (1938) observed similar changes in the spleen in *P cynomolgi* infections the maximum size being reached in one to four days after the peak of parasitaemia

Taliaferro and Mulligan (1937) came to the conclusion that in monkey malaria the soft enlargement of the spleen in the primary attack, e g in *P knowlesi* and the early stage of *P inui* infections could be accounted for by simple hyperaemia The firmer enlarged spleen of continued malaria was the result of cellular changes arising from the development of acquired immunity possibly in extreme chronic cases associated with increase in fibrous tissue The limiting factor in the size of the spleen in acute malaria e g *P knowlesi* or *P falciparum* infections was determined by the balance set between hyperplastic cellular reactions and the degenerative and necrotic processes which arrested them

Macroscopic appearances

In acute malaria the spleen may weigh as much as 800 gm It is usually dark red or chocolate in colour but after repeated attacks particularly in *P falciparum* infections it becomes much darker and may appear jet black The organ feels soft and looks more rounded than normal Deaderick (1911) described it as a bag of pulp tending to lose its characteristic contour and assuming a more spherical state than normal Gaskell and Millar found the spleens of their cases of malignant tertian malaria soft and flabby It is intensely congested and the cut surface oozes and bleeds readily in some cases haemorrhages may be found scattered over the surface beneath the capsule and in the organ tissue The capsule is tense thin and smooth and retracts from the tissue on cutting Occasionally there may be adhesions between the spleen and adjacent organs (Deaderick 1911) The consistence is greatly diminished and the substance is often soft to the point of diffluence or may be friable and pultaceous The cut surface is deep red chocolate slatey red or black depending on the amount of malarial pigment deposited in the tissues The substance appears structureless the Malpighian corpuscles may be prominent as pale

scattered dots but may be indistinguishable According to Gaskell and Millar in some cases the Malpighian corpuscles and the trabeculae are more widely separated than normal owing to the increase of pulp tissue lying in between In pernicious primary attacks there may be no appreciable pigment but in cases where infection has been persistent or repeated pigmentation may be extreme In the deeply pigmented organ the pigment is uniformly distributed and the normal structural appearances are lost except for the Malpighian corpuscles which are not pigmented and stand out distinctly against the dark background The substance does not usually contain much pigment other than haemozoin but in some cases where there has been severe haemolysis iron-containing haemoglobin products may be present and give a positive Prussian blue reaction

In more chronic cases or after recurrent attacks the spleen may be much larger than in acute malaria sometimes weighing several kilograms The colour varies from dark red to black depending upon the malarial history of the patient Deaderick (1911) states that in his experience the cut surface is dark in proportion to the age of the infection The pigmentation depends on the infecting Plasmodium being most pronounced in *P falciparum* malaria and least in *P malariae* infections (Marchiafava and Bignami 1900) The shape of the organ is better preserved than in the acute attack and the consistence is firm. The substance is pigmented and tarry and the Malpighian corpuscles are often clearly visible as grey-white spots There is little evidence of congestion and the firmness of the organ is due as will be seen partly to increase in fibrous tissue and partly to hyperplasia of certain cellular elements Some authors state that in this stage the capsule is thickened and fixed to the tissue of the organ so that it no longer retracts on section

In the late stages of chronic malaria the spleen may be contracted and fibrotic the fibrosis involving the capsule and being sometimes associated with adhesions to adjacent organs Sections of the organ may disclose extensive fibrosis throughout the substance extending from the capsule to the connective tissue trabeculae (Marchiafava and Bignami 1900 Deaderick 1911 etc)

The macroscopic appearances of the spleen in monkey malaria as in the human disease depend on the infecting Plasmodium and the history of the case In acute infections in new and old world monkeys and apes the consistency is soft the organ feeling according to Menon (1939) like a bladder The capsule is tense and thin The normal wavy wrinkled appearance is lost and the cut surface is congested and

varies in pigmentation depending on the length of the infection In *P knowlesi* infections the substance is soft and pliable and may be diffluent Slight perisplenitis has been described by Menon (1939) indicated by swelling and desquamation of the serosa and occasional threads of fibrin In the chronic stages of *P knowlesi* and *P cynomolgi* infections the substance is firm, and may be dark grey or black (Taliaferro and Cannon 1936 Taliaferro and Mulligan 1937 Blacklock and Adler 19.. Menon 1939 Aberle 1945)

Histological appearances

Parasites

Plasmodia in various stages of development are usually found in abundance in the spleen in all forms of acute malaria Most authorities report the presence of dividing forms of plasmodia in *P falciparum* infections in smears of the spleen taken at autopsy, those forms are often present in the spleen exclusively but in smears made from splenic puncture they are not always present (Yorke Murgatroyd and Owen 19.9-30)

In the examination of the organ itself Craig (1909) found the sinuses contained multitudes of plasmodia either free in erythrocytes or phagocytosed by enormous macrophages mononuclear cells and polynuclear cells in *P falciparum* infections almost every erythrocyte in the sinuses might be invaded The pulp was filled with parasitized red cells (see also Marchiafava and Bignami 1894) pigmented and sporulating forms being most frequent Free plasmodia were also present in the pulp In acute *P falciparum* infections crescents were sometimes present in the spleen after the overt malaria had lasted several days but they were never in large numbers and were often absent in cases in which they could be seen in the peripheral blood (Marchiafava and Bignami (1900) have reported precisely the opposite finding) The smaller true blood vessels of the spleen were usually filled with infected erythrocytes and contained occasional free plasmodia Dudgeon and Clarke state that thrombosis of the capillaries and arterioles was a striking feature of the spleen in their cases of malignant tertian malaria and the red cell in such vessels were very heavily parasitized

Gaskell and Millar (19.0) found very large numbers of parasites in the spleens of patients suffering from *P falciparum* cerebral malaria All stages of the parasites asexual cycle were present except those between the ring and the rosette! In the septicaemic type of *P falci*

parum infection parasites were numerous throughout the pulp and in certain degenerate areas of the pulp there were extremely dense masses of parasites A similar intense aggregation of parasites in relation to a degenerating area of the spleen has been described by Dudgeon and Clarke (1917) In this case there was a large infarct surrounded by a zone of intensely congested pulp the red cells in which were heavily parasitized although those in the infarcted region were not In the cardiac type of case (Gaskell and Millar) in which death was due to acute heart failure and in which there was usually a long history of repeated attacks of malaria parasites were present in the spleen in fairly large numbers and mostly in the ring stage of development Gaskell and Millar also described a so-called filled-in-ring form of *P falciparum* which was found lying free in the connective tissue of the splenic trabeculae

The anatomical distribution of parasites in the spleen in human malaria has not received much attention although many workers (e g Gaskell and Millar) have pointed out that parasites and pigment are rarely found in the Malpighian corpuscles It has been suggested that the spleen probably acts as a filter for removing parasitized red cells from the general circulation (Micheletti 1930) In this process the macrophages in the splenic cords probably play a particularly active part but the significance of this has been inadequately stressed in human malaria In certain forms of monkey malaria however Taliaferro and his colleagues have shown the importance of the localization of the parasites within the spleen Cannon (1941) has pointed out that in general parasites tend to localize in three organs the liver spleen and bone marrow (not all would agree to the latter—see section on bone marrow) This selective distribution occurs because of special anatomical and physiological characteristics of the organs mentioned in that in addition to capillaries and other blood vessels they possess blood sinuses which are lined by cells that are actually or potentially phagocytic In the case of the spleen the processes involved in the localization of parasites have been beautifully demonstrated in *P brasilianum* infections of Panamanian monkeys and acute *P knowlesi* infections in *M mulatta* by Taliaferro and his colleagues who found that during the crisis of the infection the parasites become regionally concentrated within the spleen where they are held in the Billroth cords and are vigorously consumed by the macrophages This active concentration and phagocytosis of parasites marks according to Taliaferro and Cannon the development of a functional acquired immunity to the parasite Such concentration is rarely described in man since the rapid degenerative

and necrotic elements of acute *P falciparum* infections tend to overshadow the more slowly developing cellular responses to infection

Pigment

Many workers have described the presence of free malarial pigment lying in the pulp cords and vessels of the spleen but the bulk of such pigment is normally found within the various phagocytic cells of the organ and remains in the cells long after all traces of parasites have disappeared Pigment is very seldom found in the Malpighian corpuscles According to Taliaferro and Mulligan (1937) the amount of pigment present in monkeys and the form in which it appears depend on the stage of the infection In early acute infections it is found in small discrete masses After the crisis or in long-standing infections it is clumped in large masses In chronic infections and relapses both forms of pigment may be found in the same cells These authors point out that this distinction between the appearance of pigment formed in acute and chronic infections has not been recognized in human malaria although from descriptions given by various authors it probably exists

Vascular system

Descriptions of the spleen in acute human malaria have been almost exclusively concerned with changes seen in *P falciparum* infections but the occasional report of the findings in other forms of acute

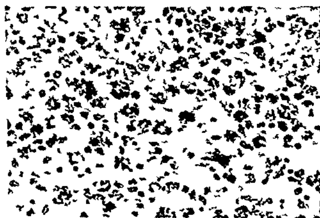


FIG 1—Spl en n n lgn t tert n l n h w ng h n o 11
(After Ash nd Sp tz 1946)

parum infection parasites were numerous throughout the pulp and in certain degenerate areas of the pulp there were extremely dense masses of parasites A similar intense aggregation of parasites in relation to a degenerating area of the spleen has been described by Dudgeon and Clarke (1917) In this case there was a large infarct surrounded by a zone of intensely congested pulp the red cells in which were heavily parasitized although those in the infarcted region were not In the cardiac type of case (Gaskell and Millar) in which death was due to acute heart failure and in which there was usually a long history of repeated attacks of malaria parasites were present in the spleen in fairly large numbers and mostly in the ring stage of development Gaskell and Millar also described a so-called filled-in-ring form of *P falciparum* which was found lying free in the connective tissue of the splenic trabeculae

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inactive in human malaria and become active only in response to an overwhelming demand on the macrophage system Taliaferro and his associates found similar failure of phagocytosis in the littoral cells of the sinuses of monkeys in simian and bird malaria They suggest that parasites and parasitized red cells are usually removed by the cells of the Billroth cords before they come into contact with the littoral cells whereas in intense infections they reach the latter and are ingested (Taliaferro and Mulligan 1937 Taliaferro and Cannon 1936 Cannon and Taliaferro 1931)

Similar suggestions were made by Marchiafava and Bignami (1900) who observed that the splenic vein often contained relatively fewer invaded red cells and free parasites than did the capillaries (sinuses) They believed that this indicated that the blood passing through the spleen became purified by the removal of parasitized cells by the macrophages of the pulp

Changes in the endothelial lining cells of the splenic blood vessels and sinuses are not often referred to in the literature Gaskell and Millar (19_0) however describe fatty degeneration in the endothelial cells in the vessels of the Malpighian corpuscles in cases of malignant tertian malaria and pigmentation and fatty degeneration in the endothelial lining of the splenic capillaries In the latter case they were probably referring to the littoral cells of the venous sinuses which are not considered to be true vascular endothelium (Taliaferro and Mulligan 1937) Gaskell and Millar stated that in malignant tertian malaria the endothelial cells which line the blood vessels are especially affected and show fatty degeneration and other degenerative changes They believed that degenerate pigmented endothelial cells were sometimes cast off from the walls of vessels and became free in the lumen Their claims have not been supported by other workers In simian malaria Taliaferro and his colleagues found neither phagocytosis nor degenerative changes in the true endothelial cells On the other hand Menon (1939) noted some endothelial swelling in the smaller follicular vessels and smaller capillaries of the marginal zone of the follicles

Proliferation of the lining cells of the large venous sinuses has recently been described by Menon (1939) and Rigdon and Stratman Thomas (194_) in *P knowlesi* infections of *M mulatta* and by Rigdon (1944) in *P lophurae* infections in ducks According to Rigdon similar proliferations have been found in *P falciparum* infections in man and *P cathemerium* infections in canaries He believes that such hyperplasia of the cells in the sinus walls represents extramedullary

malaria indicate that the picture is essentially the same as in malignant tertian (Marchiafava and Bignami 1900 Billings and Post 1915) The spleen is hyperaemic and congested the hyperaemia being increased during the paroxysm (Marchiafava and Bignami 1894 1900 Craig 1909 Deaderick 1911 Dudgeon and Clarke 1917 Gaskell and Millar 1920 Rigdon 1942 1944) Many authors have described the pulp as filled or packed with red cells without specifying clearly whether the cells were lying in the tissues or within the venous sinuses The latter are usually dilated congested and tightly packed with red cells many of which are parasitized The smaller blood vessels of the substance are congested and also distended with erythrocytes In some cases the central vessels of the Malpighian corpuscles have been found distended and congested and similar changes have been observed in the large blood vessels In chronic malaria or after a succession of acute malarial attacks the spleen is no longer congested Its size is due to hyperplastic cellular changes rather than hyperaemia In some chronic cases according to Marchiafava and Bignami (1900) degenerate or necrotic pulp may be replaced by intensely vascular tissue which may contain enormous cavernous sinuses separated from one another by thin layers of pulp or fine connective tissue containing giant cells In very long-standing cases the vascular flow through the spleen may be reduced as a result of hypertrophy of fibrous tissue

Similar changes have been reported in the spleen in monkey malaria. Taliaferro and Mulligan (1937) and other workers have found the enlargement of the spleen in acute infections to be due mainly to congestion and hyperaemia After continued malarial infection the hyperaemia is replaced by hyperplasia of cellular tissue

Although the vessels of the spleen all contain masses of parasitized red cells free parasites and malarial pigment and many kinds of phagocytic and white cells in various stages of development and degeneration there is little evidence that the true endothelial lining cells themselves act as phagocytes in human malaria This is also true of simian malaria except for occasional phagocytosis by the endothelium of the trabecular veins which (in *M mulatta*) are frequently enclosed in a thin sheath of red pulp (Taliaferro and Mulligan 1937) Phagocytosis by the littoral cells of the venous sinuses (which are true reticular cells and therefore capable of phagocytosis Taliaferro and Mulligan 1937) has been reported by several workers including Dudgeon and Clarke (1917 1919) Most authors however do not refer to such phagocytosis and Taliaferro and Mulligan deduce from this absence of comment that the littoral cells are usually relatively

(19-1) They are not mentioned by other authors e g Gaskell and Millar (19-0) They are frequently found in simian malaria particularly in acute *P knowlesi* infections Taliaferro and Mulligan agree with Craig and Deaderick that such haemorrhages probably arise from complete or partial obstruction to the splenic blood flow associated in some cases with changes in the vessel walls and perhaps with thrombosis

Degenerative and necrotic changes in the spleen have often been held to be related to vascular obstruction Areas of focal degeneration and necrosis have been observed by Marchiafava and Bignami Thayer Craig Deaderick Dudgeon and Clarke and many others Craig for instance described areas of focal necrosis similar to those seen in the liver and pressure necrosis of minute areas due to distended or ruptured capillaries Deaderick (1911) also considered these areas of cellular necrosis to result from circulatory obstruction Dudgeon and Clarke (1917) observed necrosis of the pulp cells in some cases of malignant tertian malaria and recorded similar necrosis in the Malpighian corpuscles especially pronounced in a case of blackwater fever Areas of degeneration and necrosis in both Malpighian bodies and pulp were also noted in *P falciparum* malaria by Gaskell and Millar (19-0) who regarded them as evidence of toxic activity These authors did not apparently observe any focal necrosis associated with vascular lesions

Cellular changes in the spleen

Degeneration

Generalized degeneration and necrosis of the cells of both white and red pulp of the spleen have been described in acute human malaria The lesions referred to above such as areas of focal necrosis and infarction are possibly directly related to interference with local vascular flow but other degenerative changes of a more diffuse nature sometimes occur especially in overwhelming infections such as *P falciparum* in man and *P knowlesi* in monkeys In the Malpighian corpuscles such diffuse lesions have been described as reduction in the number of lymphoid cells or even generalized atrophy and necrosis Degenerative changes have also been noted in the supporting cells pulp cells lymphocytes and even polymorphs the most pronounced changes occurring in the cells containing the greatest amount of pigment (Gaskell and Millar 19-0 Marchiafava and Bignami 1900 Dudgeon and Clarke 1917 1919) Taliaferro and his colleagues have described similar

erythropoiesis which arises in response to malarial anaemia Taliaferro and Mulligan also refer to the development of erythropoiesis in the spleen in both *P knowlesi* and *P cynomolgi* infections of *M mulatta*

Thrombosis in the splenic vessels has been described by some authorities Barker (1895) found numerous thrombi in acute fatal cases of malaria and Dudgeon and Clarke in their survey of fatal malignant tertian cases recorded that thrombosis of the capillaries and arterioles of the spleen was a striking feature The red cells involved in the thromboses were heavily parasitized and there was usually infarction necrosis or haemorrhage associated with the thrombus Deaderick (1911) does not refer directly to thrombosis but indicates that the splenic circulation becomes slowed and obstructed to the point of giving rise to oedema interstitial haemorrhage and cellular necrosis He does not indicate the origin of this slowing of the circulation but presumably refers to the congestion and distension of the vessels with packed and usually parasitized red cells white cells macrophages and giant cells Similar slowing of the splenic circulation is indicated without specific reference to thrombosis by Craig (1909) who regarded the congestion and dilatation of the venous sinuses as hindering the circulation and resulting in irregular haemorrhagic areas in the parenchyma This author noted also that the sinuses contained in addition to parasitized red cells immense numbers of phagocytes mostly large macrophages endothelial cells free pigment and parasites Embolic obstruction to the vessels due to pigment and parasitized cells has been recorded by Le Dantec (1925)

Infarction of the spleen has been described by Dudgeon and Clarke in *P falciparum* infections In one case there was a large typical infarct surrounded by intensely congested adjacent pulp containing many parasites The necrosed area contained few parasites and little pigment The state of the blood vessels is not mentioned Row Dalal and Gollerkeri (1933) and Taliaferro and Mulligan (1937) have reported true infarcts in the red pulp of the spleen in acute fatal untreated cases of *P knowlesi* infection in *M mulatta* and Bloom and Taliaferro (1938) and Hewitt (1939) in *P cathemerium* infections in canaries According to Hewitt thrombosed vessels were present in association with the splenic infarcts Rigdon (1944) has suggested that such thrombosis arises as a result of hyperplasia of cells in the walls of the larger venous sinuses and the projection of the hyperplased tissue into the lumen

Haemorrhages into the splenic pulp have been described by Craig (1909) Deaderick (1911) Dudgeon and Clarke (1917) and Pringault

increase in the pulp cells many of which showed marked division of the nucleus In malignant tertian the changes in the spleen were similar a feature being an increase in pulp cells some of which exhibited dividing nuclei Marchiafava and Bignami (1900) found the changes in chronic benign tertian malaria more noticeable in the region of the Malpighian follicles some of which were degenerate and fibrosed while others were three to four times their normal size due to hyperplasia of the lymphoid tissue The pulp cells adjacent to the hyperplastic follicles were themselves hyperplastic The presence of nucleated red cells and various stages of haemopoiesis has been reported in the spleen occasionally (Thayer 1899 Rigdon 1944)

Phagocytosis and hyperplasia in simian malaria

The following brief description of phagocytosis and cellular hyperplasia in monkey malaria has been prepared mainly from the accounts of Taliaferro and Cannon (1936) Taliaferro and Mulligan (1937) and Taliaferro (1941)

According to Taliaferro the reaction of the animal to invasion by malaria parasites is fundamentally parasiticidal and not concerned with the direct inhibition of parasitic reproduction The so-called natural immunity of the monkey probably represents the unsuitability of the host and is evident from the start of the illness showing itself in the high rate of destruction of the parasites

The resistance of the host increases as the infection progresses and eventually an acquired immunity develops which in some infections may be powerful enough to control and subdue the attack

Both natural and acquired immunity to malarial infections are associated with phagocytosis Natural immunity is displayed by phagocytosis seen most prominently in the spleen liver and bone marrow Such phagocytosis is slow and relatively ineffective The development of acquired immunity brings about a greatly increased rate of phagocytosis by individual phagocytes associated with a local increase in macrophages

As a result of their investigations in monkey malaria Taliaferro and his associates have shown that during the abrupt increase in the number of parasites in the initial stages of the disease the free merozoites and intracorpuscular parasites are phagocytosed sluggishly chiefly by the cells of the macrophage lymphoid system in the spleen liver and bone marrow At the time of the crisis in *P cynomolgi* or *P brasilianum* infections the parasitized erythrocytes appear to accumulate in the Billroth cords of the spleen, where they adhere to the local macro-

changes in acute *P knowlesi* infections in monkeys in the final stages of which the hyperplasia of lymphoid tissue is succeeded by intense cellular destruction and the follicles become denuded and atrophied (Taliaferro and Mulligan, 1937)

Phagocytosis and hyperplasia in human malaria

Taliaferro and Mulligan (1937) have reviewed the literature on this point Phagocytosis by polymorphs has been observed by some authors usually in association with necrotic changes in the tissues the cells sometimes containing pigment, sometimes parasites (Craig 1909 Gaskell and Millar 1920) True lymphocytes do not appear to be phagocytic Phagocytosis by the endothelial lining of the sinuses and blood vessels has been referred to above In some instances phagocytosis by large mononuclears or monocytes has been recorded (Thayer 1899 Pringault 1921) both in the tissues and within the sinuses (Dudgeon and Clarke 1917) Small amounts of pigment have been found in similar cells in *P knowlesi* infections in *M mulatta* either in the venous sinuses or in the splenic vessels Most of the phagocytic cells of the spleen belong to the macrophage type of cell for which there are almost as many names as there are for the authors who have described them These cells are large and usually mononuclear They are intensely phagocytic and contain pigment red cells parasitized red cells free parasites in all stages of development and often show signs of granular or fatty degeneration Their nature and origin will be described below

Most autopsy examinations of the spleen in human malaria have been made in pernicious cases so that the more active degenerative processes usually mask the hyperplastic responses of the splenic tissue Hyperplasia of the cells of the follicular lymphoid tissue has however been reported and mitoses have been observed in the cells at the periphery of the follicles An infiltration of the pulp with lymphoid cells has also been described (Thayer 1899 Pringault 1921 Dudgeon and Clarke 1919 Gaskell and Millar 1920) Mitosis has been observed in the littoral cells of the sinuses and many authors have recorded an increase in the number of pulp cells (Marchiafava and Bignami 1900 Deaderick 1911 Gaskell and Millar 1920 Pringault 1921 Rigdon 1944) Reticular hyperplasia has been reported by Levi-Valensi and Montpellier (1930) Taliaferro and Mulligan (1937)

Hyperplastic changes in chronic malaria have been described by Craig (1909) in latent benign and malignant tertian malaria In the former the connective tissue was not increased but there was a notable

sitional zone of the nodule and throughout the red pulp In the early stages of acute *P knowlesi* infections similar intense lymphoid hyperplasia is arrested by a tremendous destruction of the cells The loss of lymphocytes is often extreme and engorged macrophages frequently show degenerative changes When *P knowlesi* infections are adequately treated the histological picture in the spleen resembles that seen in *P cynomolgi* infections after the crisis except for deeper pigmentation

PATHOGENESIS

Taliaferro considers that enlargement of the spleen in acute malaria results from simple hyperaemia but this cannot be the whole explanation since on his own showing in monkeys cellular hyperplasia goes on progressively within the organ until the final stages of the disease Menon (1939) also points out that mechanical distension arising from vascular congestion cannot wholly explain the enlargement of the spleen in acute *P knowlesi* infections He quotes in this connection *the experiments of Lubarsch (1927) who showed that the size of the* spleen could not be more than doubled by maximum distension with warm saline In Menon s view other factors concerned in the enlargement of the spleen are probably cellular infiltration and swelling of the degenerated pulp cells

The circulation of blood through the normal spleen is so little understood that it is extremely difficult to interpret the congestion and dilatation of the splenic vessels in acute malaria in terms of circulatory phenomena only If the vascular changes in the spleen in malaria were initiated by the factors normally involved in other conditions of vascular collapse such as severe haemorrhage or shock the organ should be contracted and relatively bloodless rather than enlarged and congested It appears therefore that whatever happens in acute malaria in the final stage of vascular collapse some mechanism must become active in the early stages of the disease whereby the circulation of blood through the spleen becomes impeded and congestion develops What this mechanism may be is obscure Knisely (1934) has described the splenic blood flow in normal animals as a closed circuit in which the flow through the venous sinuses may be intermittent In experiments in which the transilluminated spleen of the living animal was examined he found that individual sinuses had a cycle of filling storage and emptying During the filling stage the efferent end of the sinus constricted and the vessel became distended up to 50 times its ordinary diameter with red cells which became

phages In the course of a further day or two the macrophages become extremely active and the parasitized cells are ingested rapidly and in enormous numbers This represents according to Taliaferro the beginnings of heightened immunity and once initiated continues until the parasites and invaded cells are removed and the infection is gradually subdued Superinfection with homologous strains after recovery is followed by an immediate reappearance of active phagocytosis by the macrophages The intense phagocytic activity of the crisis in acute infections or in superinfections is always associated in both *P cynomolgi* and *P brasilianum* infections with phagocytosis of uninvaded erythrocytes Taliaferro and Cannon (1936) suggest that in such circumstances the ingested cells are probably damaged

The macrophage cells immediately available for defence against malaria in the spleen are the outstretched reticular cells of the Billroth cords of the red pulp and the littoral cells of the sinuses The latter as explained above are actively phagocytic only in overwhelming infections By the time of the crisis in benign infections such as those caused by *P cynomolgi* fresh macrophages are available A few of these cells are derived from division of pre-existing engorged macrophages others are produced by division of the reticular cells of the nodules which wander into the red pulp some of the reticular cells of which also divide According to Taliaferro however by far the most important source of new macrophages is the lymphocytes Bruetsch (1932) claimed that in *P vivax* malaria macrophages arise primarily from the littoral cells of the sinuses and partly from the ordinary endothelium but Taliaferro disagrees with this and quotes the work of Maximow and Conway (1939) in favour of his view that the lymphocyte is the stem cell of the various types of macrophage which appear in the spleen in malaria Lymphoid hyperplasia is most obvious in the spleen in *P cynomolgi* and *P brasilianum* infections about the time of the crisis and shortly after Small lymphocytes disappear from the Malpighian corpuscles and are replaced by medium and large lymphocytes mitoses in both these cells and in the reticular cells become more numerous The Malpighian corpuscles increase in size in the late stages and the red pulp contains a greater number of lymphocytes and plasma cells some of which Taliaferro and Mulligan consider have migrated from the follicles All stages of transitional cells are present Taliaferro and Mulligan (1937) interpret the cellular changes in the spleen as indicating proliferation of lymphocytes in the Malpighian corpuscles and their continuous migration into the red pulp and transformation into macrophages especially in the tran-

spleen in which if Knisely's views are correct fluid is normally being continuously lost from dilated sinuses Nevertheless some true stasis probably occurs and may occasionally lead to thrombosis associated with infarcts and focal necrosis The evidence in some cases with regard to the existence of thrombosis in the splenic vessels is unquestioned but in many there is a good deal of room for doubt Many authors indeed do not refer to thrombosis at all In monkey malaria Menon (1939) found no evidence of true thrombosis Fibrin was not present in apparently obstructed vessels and Menon considered that the state of these vessels was in this respect exactly similar to those of the brain in human malaria (see Chapter IX)

Mechanical obstruction to blood flow may arise from various causes The intense phagocytic activity of the cells of the cords the massing of red cells both parasitized and unparasitized in this tissue and the swelling and hyperplasia of the cord cells must all to some extent impede the blood flow Damage to the true endothelial walls of the vessels is probably insignificant but in the sinuses the littoral cells may swell and divide and in overwhelming infections become actively phagocytic and are sometimes cast off into the lumen of the vessel causing some degree of obstruction to the blood flow Hyperplasia of the lining cells of the large venous sinuses has been described by various workers and may be accompanied as Rigdon points out by projection of the new tissue into the lumen and consequent partial obstruction Plugs of parasites parasitized cells leucocytes and phagocytes have also been described in the small blood vessels and sinuses and may cause some obstruction

All the processes referred to above which may potentially bring about obstruction to the flow of blood through the spleen are probably aggravated in the later stages of the acute disease by the agglutination of erythrocytes which takes place in the general circulation At this stage Knisely (1945) has shown that crystals probably of fibrin precipitate about the affected erythrocytes and cause the cells to become sticky not only to one another giving rise to autoagglutination but also to phagocytes He has described the circulation of small masses of agglutinated cells in the form of a sludge which resists its own passage through the smaller blood vessels The effect of such sludge in the congested malarial spleen need not be emphasized

The degenerative and necrotic changes observed in splenic tissue are as has been pointed out above either focal or general in distribution The former probably arise from acute local interference with circulation The latter also probably result from circulatory insuffi-

tightly packed presumably as a result of loss of plasma fluid through the vessel walls Retention of red cells in the sinuses lasted from a few minutes to several hours Ultimately the constricted end of the sinus relaxed and the erythrocytes escaped the sinus rapidly returning to its normal size and conducting blood like an ordinary vessel

The existence of a mechanism of this sort capable of actively obstructing the flow through the venous sinuses would offer a very satisfactory explanation for the vascular changes seen in malaria Knisely s findings have however been recently challenged by Mackenzie *et al* (1941) who claim that the circulation through the spleen is an open one and that the blood normally comes directly into contact with the cells of the pulp spaces before eventually collecting in the venous sinuses The latter and the intralobular veins into which they drain are said by Mackenzie and his colleagues to have lacunae in their walls through which fluid and red cells can pass They were unable to observe the cyclical filling and emptying of the sinuses described by Knisely but noted great variation in the rate at which blood passed through the pulp before reaching the venous sinuses They found that migrating leucocytes sometimes blocked the stigmata in the sinuses and caused a localized damming up of red cells in the pulp

Menon (1939) suggested that an acute hyperaemia is brought about in some way in malaria affecting chiefly the Billroth cords He claims that in monkey malaria congestion of the venous sinuses is by no means a constant feature In half of his 12 cases of acute *P knowlesi* infection he found the reverse was sometimes the case The meshes of the cords were packed with free blood cells and often the swelling of the pulp cords caused compression and collapse of the sinuses He points out that venous stasis should show itself as distension of the venous sinuses and a more gradual escape of cells into the pulp cords It is difficult to reconcile his findings with those of others such as Taliaferro and his colleagues who have also reported large numbers of parasitized red cells in the cords but always associated with well filled or engorged sinuses

Other factors are probably concerned in impeding the splenic circulation in acute malaria Local loss of plasma fluid may be expected in regions in which the circulation is already slowed e g in the heavily congested sinuses Some degree of stasis may therefore develop associated with complete obstruction to the blood flow in the affected vessel and interference with the oxygen supply to the related tissue Stasis is unlikely to develop readily in an organ like the

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ciency and failure of oxygenation of the tissues but many authors have attributed them to toxic elements produced by the invading plasmodia including metabolic products such as lactic and pyruvic acids The importance of such toxins and metabolites has never been established and the question remains an open one There appears to be little doubt however that the principal changes seen can be related directly to the slowing of the intrasplenic circulation and consequent anoxia of the organ

The selective phagocytosis of parasitized and non-parasitized erythrocytes *by the splenic cords* is to some extent specific since it is directed mainly against homologous strains of plasmodia Taliaferro (1941) believes that this activity is due to the presence of specific antibodies which Coggeshall (1941) suggests may sensitize the parasite and render it more susceptible to phagocytosis This does not however explain the equally avid phagocytosis of the non-parasitized red cells Brown and Broom (1935) suggest that the phagocytosis of all red cells may ultimately depend on a reduction in the electrical charge on the cell surface They demonstrated such a reduction of charge in the erythrocytes of malaria-infected untreated chicks at the time the birds began to show signs of recovery from the infection There was no demonstrable difference between infected and non-infected cells in this respect and the reduction of charge appeared to be non-specific since serum from infected chicks was capable of reducing the surface charge on normal red cells and even on the cells of other animals Brown and Broom and Findlay and Brown (1934) noted that in bird malaria the degree of splenic phagocytic activity appeared to be directly related *to the alteration in the surface charge of the red cells They claimed to* have established a direct relation between the number of spleen macrophages containing parasites and the extent to which the charge of the red cells was reduced Brown and Broom demonstrated a similar effect with solution of euglobulin and suggested that the reduction of surface charge was related directly to the appearance of humoral antibodies in the plasma Taliaferro and Cannon (1936) and Taliaferro and Mulligan (1937) have shown that in monkeys the intense phagocytosis of cells by the cords develops to a maximum in the period immediately following the crisis and they regard this as indicative of the development of acquired immunity Coggeshall (1941) states that certain immune bodies e g agglutinins first appear in *P knowlesi* infections at the point where the infection is controlled by drugs The inclusion of many immune bodies within the group of gamma globulins throws an interesting light on the experiments of Brown

and Broom and those of other authors who have reported an increase in the plasma globulin concentrations in the late stages of acute malaria

Another phenomenon of interest concerned with the increase in phagocytosis not only in the spleen but elsewhere is the precipitation of crystalline deposits on the surface of all red cells which has been observed in man monkeys and birds in the later stages of malaria (Knisely *et al* 1945 Lack 1942 Knisely 1943) The relation between this phenomenon which is non-specific and can be demonstrated experimentally in traumatic injury and the reactions observed by Brown and Broom has not been determined

Recapitulation

The enlargement of the spleen in malaria appears to be brought about by a combination of circulatory and cellular changes In the acute stages of the disease the congestive phenomena predominate but in the later stages barring a fatal issue and in chronic and recurrent malaria the cellular factors are of greater importance

The engorgement is not part of a general cardiovascular disturbance since it begins very early in the disease and is in evidence usually long before there are any indications of circulatory failure in which event in any case the spleen would be contracted and relatively bloodless The congestion must therefore be due to changes occurring in the circulation within the organ itself These have already been discussed Opinion regarding the microcirculation of the spleen is divided If Knisely s view is accepted the congestion of the venous sinuses in malaria probably arises from active constriction of their efferent extremities If Mackenzie is correct the impedance to blood flow must originate in some mechanical obstruction (possibly due to mobilized macrophages) to the escape of blood from the pulp interstices In the present state of our knowledge it is impossible to decide which view is right The blood flow is also impeded passively by swelling or proliferation of the endothelial cells and occasionally from thrombosis or plugging of small vessels with large masses of parasitized erythrocytes free parasites phagocytes and pigment In the late stages of malaria the circulation of sludged blood formed of agglutinated red cells probably also causes some obstruction

In the early stages of the infection phagocytosis is limited chiefly to the cells of the spleen liver and sometimes the bone marrow and occurs most actively in the spleen This concentration of phagocytosis arises probably from the slow blood flow through the sinuses of these organs and the direct exposure of the parasitized red cells to active

macrophages The normally sluggish circulation through the spleen is impeded in malaria by the processes mentioned above so that as the disease progresses the opportunities for phagocytosis increase Most observers agree that phagocytosis goes on mainly in the cells of the cords *and that the littoral cells of the sinuses are not greatly involved* Many have reported that the splenic arterial blood is richer in parasites than either that of the venous sinuses of the splenic vein It has therefore been suggested that the spleen functions in malaria as a filter the parasitized cells pigment and debris being removed from the circulation as the blood passes through the organ Except in overwhelming infections the cells of the Billroth cords thus remove the bulk of the parasitized cells before they reach the sinuses so that the littoral cells have few opportunities for phagocytosis This conception of the spleen as a filter has received strong support from the work of Mac Kenzie *et al* who have shown that India ink particles injected into the general circulation in mice and other animals is removed with great rapidity in the spleen where it appears on and in the pulp cells and on strands of reticulum within a few seconds of the injections

In self-limited malaria or in infections controlled in their later stages by drugs a change in the phagocytosis occurs shortly after the number of parasites begins to fall Erythrocytes parasitized and unparasitized collect in the region of the Billroth cords and after a short interval are avidly phagocytosed by the cells of the cords Both the rate and degree of phagocytosis are intensified in the individual macrophages and the process continues actively as the infection becomes subdued This selective phagocytosis is specific since once it has been developed by the Billroth cord cells it will reappear rapidly on subsequent superinfection by the homologous parasite Taliaferro and his colleagues therefore consider it an acquired immune response to the invasion This view is supported by other evidence Contemporaneously with the appearance of excessive phagocytosis specific immune bodies begin to appear in the blood amongst which are possibly opsonins which assist the phagocytosis and at about the same time (in birds) the surface charge of all erythrocytes parasitized or unparasitized becomes considerably reduced this latter phenomenon being apparently also associated with the appearance of immune bodies in the plasma *Phagocytosis may be assisted in the late stages of the disease* by the formation of crystalline deposits of fibrin on the erythrocytes described by Knisely since these deposits apparently make the erythrocytes sticky to phagocytes It is not clear what the relation is between the appearance of immune bodies and these fibrin deposits

but it cannot be specific since the latter phenomenon has been observed in conditions other than malaria such as traumatic injury

Hyperplastic changes in the spleen apparently begin early in malaria affecting chiefly the lymphocytes of the follicles and the cells of the Billroth cords At the crisis of self-limited or drug controlled malaria the hyperplastic changes are especially vigorous and are apparently related to the intense phagocytosis of this period the macrophages developing principally from the lymphocytes The stimulus for this hyperplasia may be parasitic products specific or otherwise or the more general state of anoxia arising from impedance of the blood flow In recurrent and chronic malaria the hyperplasia may continue until the organ becomes tremendously enlarged

In all infections however and especially in severe acute attacks an adverse factor (probably the progressive tissue anoxia) acts on the cells interfering with the hyperplastic response and leading to degeneration and necrosis In acute fatal infections such as those of *P falciparum* and *P knowlesi* malaria this degenerative process predominates in the final stages and may outstrip the hyperplastic activity and give rise to widespread degeneration and atrophy In *P falciparum* infections therefore the hyperplastic picture is not often clearly demonstrable

Local degenerative changes haemorrhage and infarcts are all due to vascular derangements arising in the first instance from one or othe of the various forms of circulatory obstructions which develop during the disease

The importance of the spleen in the body s response to malarial invasion is clear from the intense phagocytosis which goes on in it It appears to be one of the first lines of defence and its absence frequently leads to severe infections and high parasitaemia especially in *P knowlesi* malaria in rhesus monkeys It may also be of some importance in controlling the lysis of red cells (Stephens 1939 Fåhraeus 1939 Gear 1946

THE BONE MARROW

Changes in the bone marrow are to be expected in malaria as a reaction to the destruction of erythrocytes The picture is complicated however since extra medullary erythropoiesis is common in many infections particularly in monkey malaria and may constitute practically the whole of the erythropoietic response The changes occurring in the blood picture in all forms of malaria however give some indication of the haemopoietic activity of the tissues whether in

the bone marrow or elsewhere Such blood changes have been discussed in Chapter III and need be only briefly recapitulated here Anaemia in very acute lytic malaria is normally accompanied by few changes in the appearance of the red cells In malaria of longer duration or in cases in which there have been repeated attacks of malaria cellular changes are often observed superficially resembling those seen in pernicious anaemia Chromatophilia poikilocytosis and anisocytosis have all been frequently described Both micro- and macrocytes have been demonstrated and according to the author and the type of infection the general response has been described as either microcytic or macrocytic Nucleated red cells are not uncommon and some authors have described megaloblasts Evidence has been provided by some workers that during an acute attack of benign tertian malaria the mean corpuscular diameter is increased above normal others have found no obvious change in cell size and some in malignant tertian malaria have observed definite microcytosis Reticulocytosis has been observed by some workers during the acute malarial attack but the more usual finding is that a real increase in reticulocytes occurs only after specific antimalarial treatment has been started The absence of any degree of reticulocytosis in the peripheral blood in the acute stages of malaria has been taken by some authors to indicate a depression of bone marrow activity and by others to represent an active inhibition of the discharge of reticulocytes from the marrow into the circulation The colour index according to most authors approximates to 1 0 but some have found it persistently greater than unity in both benign and malignant tertian malaria

In acute malaria there is usually a reduction in total numbers of circulating white cells accompanied by a granulocytopenia and frequently by an increase in large mononuclear and transitional cells the latter increase is often more marked in chronic malaria or after a series of attacks The granulocytes usually show a shift to the left and primitive forms including metamyelocytes are sometimes present

The differences in the observations of various authors with regard to the blood picture in malaria can be explained to some extent by the failure to distinguish between the uncomplicated effects of malarial infection and the results of complicating factors such as nutritional deficiencies The general conclusion appears to be that the cellular elements of the blood in malaria reflect a normoblastic rather than a megaloblastic erythropoietic response associated with some depression of myeloblastic activity On the whole this view is well substantiated by the changes observed in the marrow

Macroscopic appearances

The description of the bone marrow in acute and chronic malaria given by Marchiafava and Bignami (1900) includes most of the points brought out by subsequent workers The marrow of short flat bones such as the ribs is reddish brown in colour and hyperaemic In acute infections the marrow of the long bones is little changed and retains its normal yellow fatty appearance In more prolonged infections the marrow of the upper and lower thirds of these bones changes to brownish red and in long-standing cases or after repeated attacks of malaria may become slate grey or almost black The consistence in acute cases is soft and often diffluent in chronic cases or after repeated attacks it is firm Bignami (1900) and Craig (1909) have both described the gradual replacement of the yellow fatty marrow of the long bones by vascular cellular tissue in the more chronic forms of malaria and ascribe the increased firm consistence of the marrow in these circumstances to cellular hyperplasia Taliaferro and Mulligan (1937) quote Seyfarth (1926) to the effect that under such conditions the reverse may also happen the bone marrow becoming yellower and losing both its colour and constituent cells Similar failure to react to the disease has been described by Marchiafava and Bignami who also observed a reaction in which the stimulation of functional activity was insufficient to make up for the destruction of corpuscles and in which the whole marrow of long bones became red without corresponding changes in the peripheral blood These authors in addition describe a state of amyloid degeneration of the bone marrow in severe malarial cachexia

Histological appearances

Vessels and parasites

The vessels of the active bone marrow in acute malaria are usually congested and filled with parasitized red cells Marchiafava and Bignami (1894) found that the parasites tended to be in the late stages of development and sporulating forms were common In acute stages of several days duration the same authors (1900) found that crescent forms were also present in large numbers and appeared more frequently in the marrow than elsewhere Marchiafava and Bignami (1900) and Deaderick (1911) observed many parasites lying free in the vessels, accompanied by granules and masses of malarial pigment In some cases the concentration of parasites in the bone marrow

*appears to be in excess of that found in the other organs or the peri*pheral blood but such findings are not constant and there is no clear

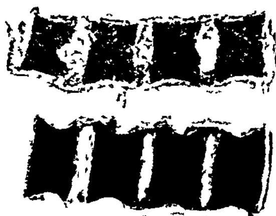


Fig 2.—Section through spinal column of patient suffering from malignant tertian malaria Section through normal spinal column shown for comparison. Note intense black pigmentation resulting from accumulation of haemozoin

evidence that any special aggregation of parasites occurs in the marrow In some cases the reverse is true For example in malignant tertian malaria Rigdon (*1942*) and Merkel (*1946*) found fewer parasites in sinuses of the bone marrow than in the vessels of other organs and Kean and Smith (1944) found parasites in the bone marrow in only 58 out of 97 cases of malignant tertian malaria with parasites in the blood or tissues

Haemopoietic tissue

In severe or inadequately treated malaria erythropoiesis is stimulated and there is often a great increase in the number of nucleated red cells (Marchiafava and Bignami 1900 Craig 1909 Deaderick 1911) Fairley and Dew (19_0) examined the marrow from fatal cases of uncomplicated and complicated acute subacute and chronic malignant tertian malaria *in Turks and Egyptians in Palestine* and observed a most remarkable erythroblastic reaction in which as many as 60 per cent of the cells in a single microscopic field were nucleated erythrocytes In some cases of pernicious malaria or after repeated infections however there may be no evidence of erythropoiesis and nucleated red cells are few (Marchiafava and Bignami 1900 Craig 1909 Paisseau and Lemaire 1916)

Fairley and Dew (1940) found that the erythroblastic response in the bone marrow in their cases was accompanied by the presence of a large number of cells of the megaloblastic type with a faintly acidophil cytoplasm. In the peripheral blood megaloblasts were frequently seen in many cases and normoblasts only occasionally. The colour index was however invariably below unity. They considered the picture very similar in many ways to that of idiopathic pernicious anaemia.

Bianchi (1940) investigated the blood and bone marrow changes in cases of acute and recently cured *P. falciparum* infections and in some post malarial anaemias. He found in acute cases a blood picture in which the colour index exceeded unity and macrocytes were constantly present associated with a bone marrow reaction which was primarily erythroblastic but in which megaloblasts were present in 11 out of 12 cases. He considered that in addition to the evident stimulation of erythropoiesis there was also some retardation of the final maturation of the red cells in the bone marrow. In recently recovered cases the picture was much the same. Post-malarial anaemia was associated again with erythroblastic hyperplasia of the marrow cells with many immature forms and typical megaloblasts. He considered that the blood picture in malaria arose partly from direct damage to the bone marrow.

Later work by Fairley and his colleagues and others has not altogether supported their earlier observations (see Chapter III) and it is now generally accepted that a megaloblastic reaction in the bone marrow is the exception and not the rule in malaria and arises as Fairley and Bromfield (1933) suggest from hyperstimulation of the marrow in response to an anoxaemia consequent on anaemia.

The bone marrow picture is thus most commonly one of compensatory hypertrophy with general increase in haemopoietic cellular tissue possibly resulting from the continuous haemolysis engendered by the disease; the bone marrow response is therefore predominantly normoblastic (Stitt 1942; Schretzenmayr 1938).

Thonnard-Neumann (1944) in a series of investigations on induced benign and malignant tertian malaria found that the bone marrow (taken at biopsy) showed intense erythropoietic activity exhibiting an increase in nucleated red cells and especially in reticulocytes. Megaloblasts were seen in only two cases one of which was a fatal *P. falciparum* infection. Lanza (1944) examined the sternal marrow in 15 cases of malaria and found similar changes. Erythropoiesis was stimulated in some cases but not in all and was normoblastic and not megaloblastic in type.

The increased erythropoiesis manifest in the bone marrow in many

cases of malaria is not normally accompanied by the expected rise of reticulocytes in the peripheral blood although these cells are frequently increased in the marrow itself Reticulocytosis normally does not develop until adequate antimalarial treatment has been administered It has therefore often been suggested that some mechanism capable of depressing medullary activity is operating during the malarial attack It is not easy to see how such depression of activity can occur coincident with obvious stimulation of erythropoiesis but light has recently been thrown on this problem by Thonnard-Neumann (1944) who found that during malaria in keeping with the increased erythropoiesis reticulocytes increased in number in the bone marrow When malarial parasites were present in the peripheral blood the discharge of the reticulocytes into the general blood stream appeared to be prevented and reticulocytosis of the peripheral blood did not occur After destruction of the parasites by specific treatment the inhibitory effect was lost and reticulocytes were discharged in large numbers into the blood Thonnard-Neumann therefore believes that the plasmodia although they do not have any depressant action on the marrow function itself can in some way interfere with the escape of reticulocytes from the marrow to the blood

Leucopoiesis

Fairley and Dew (1920) reported that in contrast to the active erythroblastic reaction seen in the bone marrow in their cases there was a complete absence of leucoblastic response Myelocytes were few and showed no mitosis Even in cases in which the patient died from bronchopneumonia complicating malignant tertian malaria there was no leucoblastic response Other authors have noted a similar failure of leucopoiesis in the marrow in malaria some have described what amounts to an aplasia (Warasi 1927 Deaderick 1911 Seyfarth 1926) Bianchi (1940) laid great stress on the contrasting picture of stimulated erythropoiesis and depressed leucopoiesis in malignant tertian malaria in Sardinia He found a leucopenia in the peripheral blood in all cases associated with a decrease in granulocytes and an increase in large monocytes the latter indicating that the derangement of leucopoiesis was primarily connected with the development of granulocytes In acute malignant tertian malaria there was clear hypoplasia of the granulocytes with a deficiency of myelocytes and premyelocytes In some cases there appeared to be some stimulation of the maturation of the myelocytic cells The same picture was seen in recently-cured cases of malignant tertian malaria and was also evident

in severe post-malarial anaemia where there was a pronounced hypoplasia of granulocytic cells although there was some hyperplasia of certain other leucocytic elements especially the lymphocytes and plasma cells Bianchi believes that these bone marrow changes must originate from the action of some soluble factor produced by the malaria parasite Regressive changes in the granulocytic cells of the bone marrow have recently been again described by Lanza (1944) in association with increase in reticuloendothelial tissue and plasma cells This author stresses the importance of such inhibition of myelogenous development in the development of malarial leucopenia

Mention has been made above of the combination in the marrow of inhibition of myeloblastic activity and increase in lymphocytes and plasma cells Taliaferro and Mulligan (1937) have reviewed the evidence concerning proliferation of such white cell element and note that increases in the following cells have been reported (i) in acute malaria various kinds of unspecified medullary cells including presumably the lymphocytes and plasma cells mentioned by Bianchi and (ii) in chronic malaria megakaryocytes lymphoid cells large cells of the vascular medullary tissue showing mitotic nuclei giant cells Atrophy of the leucocytic tissue has been described by Deaderick (1911) in chronic malaria and Seyfarth (19-6) in aplastic malarial anaemia

Phagocytosis

Phagocytosis of pigment cellular debris and intracorpuscular and extracorpuscular parasites by various cells in the marrow has frequently been described in malaria Such phagocytosis has often been noted taking place in cells lying free in the small blood vessels and sinuses Thus Marchiafava and Bignami (1900) describe globuliferous or pigmented macrophagi clinging to the vascular walls In the tissue around the vessels similar macrophages were abundant some of which were necrotic Fairley and Dew described large phagocytic cells in the marrow tissue containing ingested malarial pigment red corpuscles parasites and even leucocytes Many other authors have observed such macrophages and sometimes phagocytosis by polymorphs and even the so-called endothelial cells lining the marrow sinuses Phagocytosis of both pigment and parasites however seems to be less obvious in the bone marrow than in some other organs such as the spleen even when parasites are abundant Taliaferro and Mulligan have listed the cells observed to act as phagocytes and found them similar *to those known to behave in this way in the spleen The cells involved* are (i) the fixed and free reticular cells of the parenchyma (ii)

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the littoral cells of the sinuses which show little activity and (iii) various free cells in the lumen of the sinuses including mononuclear macrophages polyblasts (defined as cells intermediate between non granular leucocytes and macrophages) and heterophils (polymorphs)

Hyperplasia

The changes in the bone marrow in malaria have been carefully studied by Taliaferro and his associates in animals In birds infected with *P cathemerium* some erythropoietic hyperplasia occurs after acute infections have become established but the reaction is never pronounced In monkey malaria the changes depend on the host and the infective Plasmodium used as well as the length and intensity of the disease Changes most closely resembling those seen in acute human malaria are found in the bone marrow in *P knowlesi* infections in *M mulatta* In late acute untreated cases (killed) there was little hyperplasia of erythropoietic tissue in the bone marrow but various stages of polychromatic erythroblasts were found in the sinuses of the liver and spleen In fatal cases the bone marrow in different animals showed great variation in erythropoiesis In some there was considerable activity in others practically none In relapses after treatment and in fatal superinfection with heterologous strains of *P knowlesi* irregular erythropoietic hyperplasia occurred in the marrow together with ectopic erythropoiesis in many other organs There appeared to be no relation between the latter and the degree of hyperplasia of the marrow Phagocytosis was never a prominent feature in monkey malaria although enormous macrophages filled with clumped masses of pigment were common in superinfections (they were also present in the lung and kidneys)

Taliaferro and Mulligan found some degree of marrow hyperplasia and occasional lymphoid hyperplasia in long continued *P brasilianum* infections in Panamanian monkeys The latter could not be demonstrated in relation to malarial infection in *P knowlesi* infections of *M mulatta* since it occurred spontaneously in non-infected animals Phagocytosis was not a prominent feature of the marrow cells the littoral cells occasionally contained pigment and in many cases the histological picture suggested the transformation of lymphocytes and monocytes into phagocytes Phagocytosis was always more obvious in the liver and spleen In this respect the bone marrow in *P cynomolgi* infections showed a concentration of phagocytosed parasites no higher than in organs in which macrophages were not active against the parasites such as the lung kidney or heart (Aberle 1945 Nauck

1934 Taliaferro and Cannon 1936 Rigdon and Stratman-Thomas 194-)

PATHOGENESIS

The erythroblastic response to malarial infection is essentially normoblastic although in very severe cases it may apparently acquire an element of megaloblastic activity The bone marrow reaction is reflected in the blood picture which is mainly normocytic although occasionally complicated by some degree of macrocytosis and with a colour index of about unity Despite the obvious erythroblastic activity of the marrow reticulocytes are not commonly found in the blood in large numbers until the infection has been controlled by specific drug treatment This is not due to medullary inhibition since there are usually very large numbers of nucleated red cells and reticulocytes in the marrow and the numbers of the latter have been found to rise during an infection Thonnard-Neumann has therefore suggested that during the active stages of an infection there may be some inhibition of the escape of reticulocytes into the blood stream from the marrow He has produced evidence indicating that this inhibition is associated with the presence of parasites in the peripheral blood

The leucoblastic activity of the marrow is apparently partly depressed and sometimes partly stimulated Most authors agree that there is a granulocytopenia in the active disease but this appears to be associated in many cases with a shift to the left in the polymorphs and sometimes the presence of myelocytes and metamyelocytes in the blood It is difficult to understand how a shift to the left can occur in association with a depression of granulocytes unless there is some mechanism at work which allows the unusually early escape of developing granulocytes from the marrow or which as in sulphonamide poisoning inhibits maturation Bianchi has claimed that in his cases the opposite occurred and maturation of the granulocytes was accelerated but his observations on this point are unconfirmed Certain other cellular elements seem to be stimulated by malarial infection particularly the plasma cells and lymphocytes It is conceivable that the large monocytes which appear in excess in the peripheral blood are derived from the latter

The erythroblastic changes in the marrow are presumably the physiological response to the continued loss of red cells taking place during the infection Megaloblastic activity may indicate when it occurs some more direct effect of the parasite or its products

The state of anoxia existing in the marrow as a result of the anaemia and other general processes affecting the circulation and the oxygenation of the blood may also influence the haemopoietic reaction According to van Liere (1942) Dallwig *et al* (1915) have demonstrated erythropoietic stimulation in the marrow following exposure to a 14 per cent reduction in atmospheric oxygen and other workers have shown that under similar conditions of anoxia an increase in lymphocytes occurs in the blood at the expense of the polymorphs Meyer *et al* (1935) found that rats and guinea pigs exposed to persistent moderate anoxic anoxia first showed a leucocytosis and subsequently a leucopenia which they considered arose from depression of the lymph nodes and bone marrow

It has often been said that the bone marrow because of its sinuses and sluggish circulation acts as one of the main centres of phagocytosis in the body in malaria infections As will be seen from the text there is little justification for this claim either in human or simian malaria

There is an interesting lack of evidence concerning plugging thrombosis or stasis of the marrow vessels in malaria Congestion is common but there seems to be no other mechanical obstruction to blood flow This is probably due as in the case of the liver to the normally relatively permeable nature of the endothelium and littoral cells of the sinuses which allow free passage of fluid from the blood under physiological conditions and are thus little affected in this respect by anoxia

CHAPTER XI

THE ADRENALS AND HEART

ADRENALS Clinical Algid malaria Pathological Changes Microscopic appearances — Histological changes General picture Phagocytosis [illegible] Pathogenesis Similarity of algid malaria and syndromes of adrenal toxicity and Addison's disease — Adrenal anoxia
HEART Clinical Myocardial failure — Cardiac involvement in malaria Pathological Changes Microscopic appearances — Histological changes Parasites Vascular and degenerative changes Pathogenesis Circulatory changes — Degeneration of cardiac muscle — Action of haematin

ADRENALS

CLINICAL EVIDENCE OF ADRENAL INSUFFICIENCY IN MALARIA

The similarity between the clinical symptoms of certain forms of pernicious malaria and those of adrenal insufficiency has been remarked by many workers For instance Paisseau and Lemaire (1916) pointed out that asthenia and low blood pressure were cardinal signs in certain forms of algid and comatose malaria and in a series of such cases were able to demonstrate severe lesions in the adrenal glands Other authors as we shall see have made similar observations and it has consequently been frequently argued that there must be a causal relation between the adrenal lesions observed and the symptoms encountered clinically in pernicious malaria

Recently however it has been shown that a syndrome very closely allied to traumatic shock may develop in malaria independently of adrenal damage (Rigdon 1942 Kean and Taylor 1946) The symptoms of traumatic shock are very closely similar to those of adrenal insufficiency (Swingle *et al* 1933) Moreover shock and associated phenomena such as tissue anoxia may give rise to pathological changes in the adrenal glands and will cause humoral changes identical with those of adrenal insufficiency e g an increased potassium and decreased sodium plasma concentration It thus becomes extremely difficult to decide precisely the role of the adrenal in the pathogenesis of malaria even in cases in which the clinical and pathological findings are highly suggestive of adrenal insufficiency Nevertheless for many years information has been accumulated on this point and there is abundant evidence of pathological change in the adrenal glands and

of clinical signs of apparent adrenal insufficiency The question of which is the primary condition the adrenal failure or the prevailing state of vascular collapse will not be discussed here (see Chapter XII)

According to Flosi (1944) Valenti in 1907 was the first physician to suggest that adrenal insufficiency might play an important part in the clinical manifestations of severe acute pernicious malaria Other workers confirmed his views and Brun described not only acute but chronic signs and symptoms attributable to adrenal dysfunction and suggested that the pathological changes responsible for such deviations in function might arise from deposition of malarial pigment in the adrenals or from accumulation of parasitized red cells in the vessels of the glands

Paisseau and Lemaire (1916) reported as already mentioned the appearance of certain symptoms of adrenal failure in pernicious malaria particularly in algid and comatose patients and in some cases were able to correlate the clinical findings with pathological changes in the adrenals In three cases the lesions in the adrenals were much more pronounced than those in other organs such as the liver and were considered by the authors to be responsible for the clinical condition Paisseau and Lemaire pointed out the similarity between some of the adrenal symptoms of malaria and conditions arising in other diseases such as diphtheria scarlet fever and typhoid fever Such symptoms were especially obvious in pernicious comatose or algid malaria Ces etats se presentent en effet avec une symptomatologie presque purement surrenale et peuvent etre consideres comme de veritables surrenalites palustres aigues An adrenal syndrome of a more chronic type sometimes occurred associated with pigmentation of the skin and mucous membranes and identical in many respects with the classical picture of Addison s disease Some evidence of adrenal insufficiency could be found in many different forms of malaria the principal clinical signs being asthenia hypotension peripheral vascular failure and digestive disturbances giving rise to abdominal and lumbar pain

Paisseau and Lemaire distinguished fulminating (suraigue) acute and subacute forms of adrenal insufficiency in malaria The fulminating cases developed suddenly as a rule and often without warning *Un homme faisant partie d une troupe en marche tombe brusquement sur la route un blesse entre dans le coma sans raison apparente* The patient shows no signs of central nervous system involvement His muscles are relaxed but not paralysed reflexes are unchanged The temperature is at first elevated the blood pressure slightly lowered

the pulse full and bounding In the course of a few hours the temperature falls to normal and below the blood pressure becomes very low and the pulse feeble and soft Death occurs after a few hours This form of coma can be clearly differentiated from that of the cerebral type of malaria in which there is abundant evidence of involvement of the central nervous system

The algid form of pernicious malaria similarly presents elements which may be considered primarily adrenal in origin e g profound asthenia and reduction of bodily temperature The pernicious symptoms often develop during an apparently uncomplicated malarial attack and are ushered in by a falling temperature and often signs of nervous instability and delirium and occasionally vomiting The temperature begins to fall rapidly to normal and below and algid collapse ensues The appearance of the patient is characteristic He lies still in a state of profound collapse face drawn eyes sunken and fixed The skin is pale and cold and covered with clammy sweat The pulse is small and soft and a little faster than normal The blood pressure is very low There may be severe epigastric pain and intractable vomiting and diarrhoea accompanied by agonizing muscular cramps Sometimes the gastro-intestinal symptoms may predominate and loss of fluid may become extreme the patient passing into a choleraic state Death occurs suddenly When recovery takes place the temperature rises as the blood pressure improves

Severe signs of what Paisseau and Lemaire regarded as adrenal failure may also develop in the course of more prolonged malarial attacks especially in cases in which the temperature is high and there has been the development of pronounced anaemia The temperature suddenly falls to normal or below and diarrhoea and vomiting develop The patient complains of severe abdominal pain He is emaciated and anaemic and asthenia is the predominating symptom He is prostrated with soft small pulse and extremely low blood pressure Reflexes are feeble but present and he is mentally clear The asthenia may develop and progress rapidly and death is likely to occur after some days The condition is thus less acute than that described above Sometimes after recovery from the severe condition the patient may pass into a condition resembling Addison s disease

Subacute adrenal symptoms are also described by Passeau and Lemaire developing in apparently relatively mild cases In these states the patient is emaciated anaemic complains of nausea and may vomit The blood pressure is low Occasionally these cases develop pigmentation of the skin and mucous membranes as in Addison s disease In

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with humoral changes similar to those found in Addison's disease One of their cases showed extensive pigmentation of the skin

Maciel (1940) reviewed the literature to that date and described 20 cases of malignant tertian malaria in which evidence of adrenal insufficiency in some form could be demonstrated He came to the conclusion that the adrenals were affected in malaria as frequently as any other organ of the body including the spleen and liver Adrenal insufficiency probably influenced considerably the general reaction of the body to the malarial invasion

The above summary of the literature on the clinical evidence of adrenal involvement in malaria is very incomplete Those interested should consult the book recently published by Flosi (1944)

It will be seen that the clinical evidence is based largely on the development either acutely or more slowly of a syndrome which includes asthenia lowered arterial blood pressure and peripheral vascular failure with skin pigmentation abdominal cramps etc in some cases To some extent the proposition that such symptoms and signs are related to adrenal insufficiency has been supported by chemical observations on the blood and tissues made during the pernicious malarial attack In estimating the value of such chemical evidence however it must be realized that conditions other than adrenal insufficiency may give rise to them and that such conditions e g the state of shock and anoxia may accompany all severe forms of malaria It is thus extremely difficult to accept the evidence at its face value in any given case either for or against the involvement of the adrenals

We have already discussed some of the biochemical evidence in other contexts (see Chapter IV) where it is more relevant Thus Miyahara (1936) and others have observed hypochloraemia in acute malignant tertian malaria and various changes in the blood glucose concentration (both hypo and hyper-glycaemia) during the paroxysm have been described Both the changes in chloride and sugar content of the blood could be explained on the basis of alterations in adrenal function and in this connection the experiments of Chessa (1938) may be of some importance This author found that in about one-third of his cases of acute and chronic benign and malignant tertian malaria some of which showed signs of adrenal insufficiency there was a definite increase in sensitivity to insulin He deduced from this that there was in these cases some degree of adrenal dysfunction possibly also associated in some with derangement of the thyroid and liver He explained his results in chronic cases by suggesting that the adrenal lesions once produced recovered only slowly

some in addition to adrenal symptoms there may be clinical features referable to damage of other organs such as the liver and bone marrow Examples of such complicated syndromes are seen in malarial cachexias of various kinds in which emaciation develops rapidly and cachexia is progressive and fatal In three such cases the authors found lesions of the adrenals and severe *lesions in the liver spleen bone marrow* and intestines

Cases such as those described by Paisseau and Lemaire are uncommon to-day but were reported by many observers during the war of 1914–18 Monier Vinard and Teysonnieres both described cases quoted by Paisseau and Lemaire and Garin *et al* (1917) noted adrenal syndromes in 24 out of 590 cases of malaria of all the common types The syndrome in these cases developed insidiously The clinical picture was one of *pallor malaise asthenia* great loss of weight and bronze *discoloration of the hands face and mucosa* of the mouth

Dudgeon and Clarke (1917) investigated the cause of sudden death in cases of malignant tertian malaria in troops and found changes in the adrenals in most cases They considered that such pathological lesions were associated with the vascular collapse which was the striking clinical feature of the fatal cases

Fraga and Motta (1917) came to similar conclusions in regard to the *importance of adrenal failure as a cause of* death in malaria and in the same year MacDowell suggested that the three symptoms asthenia hypotension and bradycardia which were often found in malaria might have their origin in adrenal insufficiency and associated sympathetic hypotonus

Many other authors reported evidence of adrenal failure in malaria *both clinical and pathological (Flosi 1944) but the first* experimental investigation of the problem was conducted by Natali (1934) who reported the clinical and anatomical findings in a case of *P falciparum* infection and in a number of non-immune monkeys infected with *P knowlesi* He found constant and characteristic changes in the adrenals and claimed that such lesions could account for the severe symptoms and rapidly *fatal course of the disease in both man and* monkeys The changes in the adrenals in severe cases predominated over those in all other organs—a finding which has not always been confirmed Natali believed that the lesions in the adrenals were produced by the action of a malarial toxin

Peregrino and Brandao (1937) reported adrenal insufficiency (i e asthenia low blood pressure etc) in four cases of malaria associated

the sodium plasma concentrations There was also a general clinical improvement Sodium salts (chloride citrate and bicarbonate) also relieved the clinical picture and adjusted the electrolyte concentrations When desoxycorticosterone was given during the period of clinical activity of the malaria the excessive rise of potassium concentration during the paroxysm was checked and the blood pressure tended to rise Administration of either desoxycorticosterone or sodium salts significantly shortened the length of convalescence

Flosi s results are particularly interesting in that they show that the clinical development of adrenal symptoms in malaria may sometimes be checked by the administration of adrenal cortical extracts or sodium salts This may be taken to indicate the existence of adrenal insufficiency elements in the symptom complex of the disease The interpretation of his findings with regard to electrolyte concentrations in terms of adrenal changes however is not so obvious as he suggests

PATHOLOGICAL CHANGES

Macroscopic appearances

Paisseau and Lemaire (1916) divided the lesions observed in the adrenal glands in acute malarial cases dying in coma or from algid complications into two groups i e (i) those in which the predominant change was one of degeneration and necrosis and in which there was little congestion or haemorrhage and (ii) those in which congestion and haemorrhage were the most conspicuous changes The degenerative type of gland was not atrophied or congested The necrosis was present mainly in the inner zone of the cortex and the whole organ was paler than normal the bright yellow colour of the cortex was lost and replaced by a uniform dull grey yellow representing loss of lipoid substances The pigmented zone was paler than normal In the haemorrhagic and congestive type of lesions the glands were deeply engorged and the cut surface displayed many small bleeding points and scattered areas of frank haemorrhage The organ was reddish grey throughout and there were many distinct areas of necrosis chiefly confined to the inner zones of the cortex

Dudgeon and Clarke (1917) in their investigation of cases of malignant tertian malaria dying in acute cardiovascular failure found that there was commonly a diminution in the amount of lipoid substances in all layers of the cortex resulting in a distinct loss of the normal yellow coloration In addition in some cases there were areas of extensive necrosis of the gland substance in both cortex and medulla

Study of other inorganic elements of the blood in malaria has also provided evidence which could be interpreted in terms of adrenal insufficiency Thus a raised plasma potassium concentration has frequently been observed in animal and human malaria and it is generally agreed that the concentrations observed are in excess of those which could be accounted for by the destruction of red cells as a result of plasmodial invasion The potassium must therefore come from the tissue cells and Zwemer Sims and Coggeshall (1940) have pointed out that since the ratio of intra/extra cellular potassium is dependent on the proper functioning of the adrenals loss of intra-cellular potassium may indicate some change in adrenal activity They suggest that in addition the high potassium plasma concentrations found in malaria may in themselves be toxic to the adrenal glands

Flosi (1944) recently investigated the occurrence of adrenal signs and symptoms in acute *P falciparum* *P vivax* and mixed malarial infections He found that the incidence of such symptoms was not very great but in nine out of twelve selected cases there was clinical evidence of adrenal insufficiency In eight of these cases in all of which *P falciparum* was the infective agent either alone or in association with *P vivax* he observed a persistently raised plasma potassium concentration and a reduced sodium plasma concentration after antimalarial treatment In the ninth case these changes in electrolyte concentration were manifest only during the administration of a salt deficient diet In two other cases infected with *P vivax* without clinical signs of adrenal disturbance an increase of potassium and reduction of sodium plasma concentrations occurred during the malarial paroxysm normal concentrations being restored in the apyrexial intervals Flosi calls this temporary change in potassium and sodium concentrations disionia paludica and considers it related to the destruction of red cells at the time of sporulation of the parasites and not to changes in the adrenal glands The latter however are probably present in cases which exhibit alterations of potassium and sodium concentrations during the paroxysm and in which these concentrations do not return to normal in the apyrexial period

Following antimalarial treatment persistent symptoms of adrenal insufficiency in Flosi s cases were progressive asthenia anorexia loss of weight and low blood pressure Restriction of sodium chloride in the diet resulted in exacerbation of these symptoms and associated changes in plasma electrolyte concentrations Administration of desoxycorticosterone acetate to cases of presumed adrenal insufficiency caused a rise in blood pressure and a fall of the potassium and rise of

zona reticulosa and bounded by the medulla The cell columns were completely disintegrated by the haemorrhages and the dilated vessels and extensive degeneration and necrosis of the parenchymal cells was found in association with the haemorrhagic areas There were often considerable areas of necrosis in which the whole tissue was disintegrated and replaced by cytoplasmic and nuclear debris These necrotic areas were often more extensive than those the authors observed in the purely degenerative type of glandular lesions The normal pigmentation of the cells was lost

Paisseau and Lemaire considered that the necrotic areas arose primarily as a result of thrombosis of associated vessels but beyond referring to such thrombosis in general terms they did not produce much positive evidence of its existence

In both degenerative and haemorrhagic lesions parasitized red cells were very numerous and in some cases appeared to pack the capillaries

Lesions in other organs were not conspicuous which in the author s view established the pathogenic role of the adrenal changes particularly as in other cases examined (e g blackwater fever) in which clinical evidence of adrenal insufficiency was lacking lesions of the adrenals were not obvious

Histological changes similar to those described by Paisseau and Lemaire have also been observed by other workers in fatal cases of acute malaria associated with apparent clinical evidence of adrenal insufficiency Most other authors however have described a type of lesion which includes elements of both those described by Paisseau and Lemaire Thus Dudgeon and Clarke found a constant reduction in lipoid content in all layers of the cortex and a reduction in chromaffin tissue in the medulla Malarial pigment was present in the endothelial cells of the sinuses and in macrophages lying in the sinuses The authors also refer to such pigment as being present in the parenchymal cells This was also noted by Thayer (1899) but has not been reported by other observers There was extensive necrosis of the parenchymal cells in both cortex and medulla and the blood vessels of the organ were congested In some deeply congested glands there were also haemorrhages into the substance The cortical tissue showed marked granular degeneration and vacuolization of the cytoplasm of the parenchymal cells In some cases the degenerative changes were confined to scattered areas which appeared to be associated with the presence of vascular thrombosis The vascular congestion and haemorrhage varied considerably in degree and position in different glands in some the cortex was primarily affected in others the

and congestion of blood vessels in both In the more deeply congested glands small haemorrhages were common throughout the gland substance

Natali (1934) described the adrenals in a single case of acute malignant tertian malaria from West Africa He found oedema of the capsule and dilated and congested blood vessels throughout the gland There were areas of necrosis in both the cortex and medulla particularly concentrated in the inner layers of the cortex

These authors all refer to extensive damage to the adrenal glands in cases of malaria associated with clinical signs suggestive of adrenal involvement but many other workers have either not observed such lesions in autopsies of clinically similar cases or have failed to refer specifically to the adrenal glands in their reports It is fair to assume in the latter case that lesions of the glands if present cannot have been conspicuous It appears therefore that changes in the adrenal glands are not constantly present in acute malaria

Histological changes

Paisseau and Lemaire (1916) described the histological pictures of the degenerative and haemorrhagic lesions separately In the degenerative gland the spongiocytic cells had generally disappeared from the cortical layers The parenchymal cells of the zona reticulosa were pale and degenerate the degeneration ranging from simple granular changes to advanced necrosis with pyknotic homogeneous or broken up nuclei In some cells there was a clear zone of plasmolysis in the nuclear region The cell columns were broken up and individual cells were often separated and completely disintegrated Practically all the cells in the gland were involved to some degree but the necrosis was most conspicuous in the glomerular and reticular zones In many areas the damage was so extensive that the tissue resembled an amorphous mass of debris In the fascicular zone the normal pigmentation was frequently lost Degenerative lesions also occurred in the medulla but these were less extensive and less developed than those in the cortex The blood vessels were not grossly involved and there was no appreciable congestion Cellular infiltration was common in the cortico-medullary junction zone

In the haemorrhagic type of gland there was intense congestion of the venous vessels The cut surface was covered with small haemorrhages and the vessels of both cortex and medulla deeply congested and thrombosed The haemorrhagic zone was on the whole concentrated mainly in the inner half of the cortex involving especially the

similar lesions in the adrenals although all are agreed that in the non-immune and untreated animal the disease is rapidly fatal Rigdon and Stratman-Thomas (1942) for instance carried out careful autopsies on 26 maccacus monkeys infected with *P knowlesi* All these animals either died of the disease or were killed in the acute stages Gross pathological changes were seen only in the liver and spleen and pathological changes in the adrenals were relatively inconspicuous consisting of scattered small haemorrhages seen in only a few monkeys mostly in the region of the cortico-medullary junction The vessels of the glands were congested and filled with parasitized red cells They concluded in disagreement with Natali that although adrenal lesions might be of importance in human malaria they could be of little significance in monkey malaria since they were so seldom found Taliaferro and Mulligan (1937) do not refer to changes in the adrenals in acute *P knowlesi* infections in *M mulatta* but in cases in which a fatal relapse occurred after successful treatment of the primary attack they mention general pathological changes in various organs including degeneration of the parenchymatous cells of the suprarenal

According to Taliaferro and Mulligan (1937) there is not much evidence of extensive phagocytosis of pigment or parasitized erythrocytes in the adrenal glands in malaria Reference has been made above to the findings of Dudgeon and Clarke who reported phagocytosis by macrophages the endothelial lining cells of the sinuses and occasionally by the parenchymal cells themselves Others have recorded phagocytosis by macrophages or polymorphs (Pringault 1921 Thayer 1899) and by the littoral cells of the sinuses On the whole however phagocytosis does not appear to be a prominent feature of the adrenal s reaction to malaria in either human or monkey hosts Taliaferro and Cannon (1936) for instance found that the macrophages in the adrenals of Panamanian monkeys or *M mulatta* played little part in the defence against acute *P brasilianum* or *P knowlesi* infections respectively Thus in the late stages of acute *P knowlesi* infections although parasitized red cells were extremely common in the blood vessels of the adrenals phagocytosis was represented by at most only a few small discrete granules of pigment in a few monocytes or small polyblasts (Taliaferro and Mulligan 1937) In chronic infections as in infections of splenectomized animals some phagocytosis was observed in the littoral cells of the sinuses For instance in one monkey which died from an acute relapse of *P knowlesi* malaria following the successful control of the primary attack the littoral cells contained considerable amounts of pigment

medulla Parasites were present in the red cells in most cases but the degree of parasitization of the blood varied greatly from case to case

Necrosis of the parenchymal cells of the adrenals in human malaria and thrombosis of the vessels of the glands presumably accounting for the degenerative changes of the tissue have also been described by Wenyon (19-2) and Barker (1895)

As mentioned above however other workers have not been impressed with the appearance of adrenal changes at autopsy in malarial cases Thus Pringault (1921) reported some degree of vascular congestion but made no reference to degeneration although in one case there was some proliferation of perivascular connective tissue Taliaferro and Mulligan (1937) quote Levi-Valensi and Montpellier as failing to find noteworthy lesions in the adrenals in three autopsies of malaria cases

Natali (1934) examined the adrenals in one case of *P falciparum* infection and in eight monkeys which were superinfected with *P knowlesi* He concluded that in acute malaria anatomical changes in the adrenals could arise independently of concomitant changes in other organs and considered that such lesions were responsible for the rapidly fatal issue of algid and comatose malaria The severity of the pathological changes seen was apparently independent of the degree of parasitaemia observed in the glands at autopsy or of the stage of parasitic development The lesions were fundamentally haemorrhagic and necrotic in type and affected chiefly the zona fasciculata which was the centre of necrotic damage and the zona reticulosa which suffered chiefly from haemorrhage and congestion An inflammatory response on the part of the host referred to by Paisseau and Lemaire as early cellular infiltration of the cortico-medullary region was present in the form of a diffuse infiltration of the tissues with lymphocytes histiocytes and plasma cells

Natali considered that the degenerative and necrotic changes in the parenchymal cells could not be considered specific to the malarial infection but were probably similar to those seen in other degenerative inflammations of the glands and exhibited the basophilic granular degenerate changes in the cytoplasm characteristic of such inflammations He defined the malarial lesions of the adrenal as being a conglomeration of degeneration necrosis haemorrhage vascular congestion and thrombosis cellular infiltration and oedema He believed that the lesions arose primarily as a result of the activity of a malarial toxin

By no means all observers of acute monkey malaria have remarked

rise in blood non protein nitrogen but this again has been frequently demonstrated by others in terminal infections

It seems reasonable to suppose therefore that adrenal insufficiency may arise during the course of a malarial infection but before attributing the symptoms described above to failure of the adrenal glands due allowance must be made for the fact that malaria is a general disease in which the attack is felt by many organs besides the adrenals Derangement of the glucose concentration of the blood for instance might arise from liver dysfunction Similarly a rise in urea nitrogen might result from renal failure and so on The great difficulty with regard to the adrenals in malaria appears to be not so much to determine whether the glands are involved as to decide what part adrenal dysfunction plays as a primary factor in producing the clinical picture of acute pernicious malaria As was pointed out above although there is a good deal of evidence to show that in certain cases pathological changes occur principally in the adrenals to the exclusion of other organs and are severe enough to damage the gland and interfere with its function there is also a considerable amount of negative evidence particularly in monkey malaria which indicates that the signs and symptoms of adrenal failure may occur without corresponding pathological changes in the glands If we are to suppose that adrenal insufficiency occurs in malaria we must therefore find an explanation for such functional deviations with or without anatomical changes in the glands

Paisseau and Lemaire ascribe the changes they observed in the adrenal glands to the activity of a soluble malarial toxin but such a factor has never been identified The pathogenesis of the degenerative type of lesion they describe is extremely difficult to envisage although the pallor of the organ and absence of congestion suggest some interference with the blood supply The other lesion in which congestion and haemorrhage predominate and which is commonly described by other workers is easier to understand since such visceral congestion is the general accompaniment of the type of circulatory failure which is a characteristic feature of the clinical conditions of comatose and algid malaria described above (Moon 1938) The congested state of the organ indicates a slowing down of the circulation through it and would lead as in other organs (e g the brain) to concentration of the cellular elements of the blood due to loss of plasma fluid to the tissues Stasis with complete temporary obstruction of the circulation through the affected vessel would follow in severe cases and produce lesions very similar superficially to thrombosis such as described by Paisseau and Lemaire The existence of true thrombosis would complicate matters

An interesting change described by Taliaferro and his associates is the development of extramedullary erythropoiesis in the adrenals in both *P cynomolgi* and *P knowlesi* infections (Taliaferro and Mulligan 1937 Aberle 1945)

PATHOGENESIS

Complete removal of the adrenal glands in an animal gives rise to a rapidly fatal condition in which the cardinal signs are anorexia vomiting diarrhoea asthenia and prostration and rapid loss of weight These signs are associated with a considerable fall of body temperature and an appreciable reduction in the basal metabolic rate a fall in chloride and in total plasma base due mainly to loss of sodium and a rise in plasma potassium and non-protein nitrogen This condition can be relieved by the administration of either adrenal cortical extract or sodium salts In man adrenal insufficiency gives rise to the syndrome of Addison s disease characterized mainly by asthenia low blood pressure vomiting and gastro-intestinal disturbances lowered metabolic rate and subnormal body temperature hypochlorhydria hypoglycaemia a fall of plasma sodium and a rise of plasma potassium and the appearance of pigmentation due to deposition of melanin in the skin and mucous membranes Improvement may result from administration of adrenal cortical extracts or sodium salts Exacerbation of the symptoms is produced by a low salt intake

The similarity of the above symptoms and signs to those of algid malaria is very striking For instance Paisseau and Lemaire describe a malarial condition in which the chief features are asthenia low blood pressure peripheral vascular failure and digestive disturbances associated in the more chronic states with brown pigmentation of the skin and very considerable loss of weight Flosi found similar syndromes in cases infected with *P falciparum* and furthermore found that the symptoms were aggravated by restriction of salt intake (the provocative tests of Wilder (1938) and Harrop (1933)) and were often relieved once the malarial infection has been treated by the administration of desoxycorticosterone or sodium salts The biochemical features of the blood were also in many ways similar to those of adrenal insufficiency the principal changes being a rise in potassium and fall in sodium plasma concentrations In his series Flosi was not able to demonstrate hypoglycaemia but as will be seen in Chapter IV a fall of blood sugar has been described by numerous other authors during the paroxysm and in the later stages of fatal infection Flosi also failed to detect any

rise in blood non-protein nitrogen but this again has been frequently demonstrated by others in terminal infections

It seems reasonable to suppose therefore that adrenal insufficiency may arise during the course of a malarial infection but before attributing the symptoms described above to failure of the adrenal glands due allowance must be made for the fact that malaria is a general disease in which the attack is felt by many organs besides the adrenals Derangement of the glucose concentration of the blood for instance might arise from liver dysfunction Similarly a rise in urea nitrogen might result from renal failure and so on The great difficulty with regard to the adrenals in malaria appears to be not so much to determine whether the glands are involved as to decide what part adrenal dysfunction plays as a primary factor in producing the clinical picture of acute pernicious malaria As was pointed out above although there is a good deal of evidence to show that in certain cases pathological changes occur principally in the adrenals to the exclusion of other organs and are severe enough to damage the gland and interfere with its function there is also a considerable amount of negative evidence particularly in monkey malaria which indicates that the signs and symptoms of adrenal failure may occur without corresponding pathological changes in the glands If we are to suppose that adrenal insufficiency occurs in malaria we must therefore find an explanation for such functional deviations with or without anatomical changes in the glands

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arising immediately the oxygen supply to the parenchyma begins to fail When circulatory failure is rapid and progressive functional changes develop rapidly and are followed by degenerative and necrotic anatomical changes and a vicious circle is established whereby the circulatory derangement is exacerbated by the effects of glandular dysfunction A state of anoxia in the adrenal glands thus affects not only the local tissue but all other organs normally influenced by the adrenal secretions of the glands The effects of such anoxia have been studied in animals suffering from artificially induced anoxic anoxia At first there is often a transient stimulation of the adrenal medulla with the production of excess adrenalin which produces its characteristic effects on other organs e g the liver with the mobilization of glucose from glycogen and consequent hyperglycaemia As anoxia develops the adrenal glands become exhausted and signs and symptoms of cortical failure begin to appear (Cannon 1922 Grollman 1936) For instance Giragossintz and Sundstroem (1937) have shown that rats when kept for some time in an atmosphere containing a low *oxygen concentration eventually develop a syndrome similar to that* produced by adrenalectomy and which can be relieved by the administration of adrenal cortical extracts Prolonged anoxia gave rise to cellular damage congestion and haemorrhage in the gland substance Emerson and van Liere (1938) reported a similar reduction of medullary efficiency in cats after exposure to low oxygen concentrations for some hours the adrenalin output of the adrenal falling by 40 per cent from normal

It is clear that the development of anoxic conditions in the adrenals will give rise to symptoms of adrenal insufficiency in animals and there is little doubt that similar symptoms would arise in man under the same conditions Hence with or without gross anatomical changes in the adrenals in malaria where all the elements for the production of gross anoxia of the tissues exist it is not surprising that signs of insufficiency of the adrenals are exhibited

HEART

CLINICAL

In acute uncomplicated malaria the heart is seldom seriously affected Sprague (1946) reviewing observations made on some thousands of cases of malaria seen in the South Pacific area during *the 1939–45 war found no clear-cut case of malarial heart disease* Electrocardiographic examinations of some of these cases revealed

still further but evidence of this is not very convincing so far as can be ascertained no careful histological studies of lesions have been made with a view to identifying fibrin in the apparently obstructed vessels Either thrombosis or stasis if continued sufficiently long would impede the circulation to the point of producing a state of complete anoxia in the tissue dependent for its oxygen supply upon the particular vessel concerned and changes in the local parenchyma would result first in degeneration and finally in necrosis As in other organs the anoxia itself would give rise to physiological changes in the vessel wall resulting in diapedesis of red cells and possibly later in frank haemorrhage In the later stages of the disease further vascular obstruction leading to enhanced anoxia might develop from the formation of sludged cells as described by Knisely (1945) Finally obstruction to the blood flow through the adrenals might arise from changes induced in the vessel walls including swelling and phagocytic activity of the littoral cells of the sinuses From all accounts however such changes are uncommon

Once the blood flow through the adrenal glands had been slowed and obstructed in this manner degenerative lesions would develop rapidly and interference with adrenal function would result giving rise to physiological effects including hypotension which would tend to aggravate the general circulatory changes

In cases in which no obvious lesions of the adrenal glands are found although the clinical features indicate some adrenal dysfunction it is possible that changes in the glandular circulation similar to those described above take place without complete vascular obstruction so that the final stages of degeneration and necrosis of the parenchyma do not develop the oxygen supply to the cells being reduced but not totally abolished

From the clinical and pathological evidence available it is not possible in most cases to decide whether the adrenal insufficiency was developed suddenly and violently enough to precipitate a general circulatory crisis or whether the vascular failure arose from generalized increasing loss of circulating plasma fluid resulting from injury to the vascular endothelium and the adrenal changes arose secondarily to the vascular failure In view of the changes in other organs and the relatively frequent absence of changes in the adrenals the latter possibility appears the most likely If this argument be accepted therefore it can be taken that the lesions found in the adrenals like those of most other organs in malaria develop primarily from circulatory disturbances derangement of the functional activity of the gland

symptoms were considered to result from cardiac failure although in most cases the description given by the authors is much more that of generalized vascular collapse In another group of patients described as septicaemic the outstanding sign was a very high content of parasites in the blood and tissues and noticeable degenerative changes in the heart muscle Fatal cases in this group in many of which the predominating signs were cerebral did not show marked symptoms of heart failure until just at the end of the attack

Evidence of cardiac failure or impaired cardiac function is sometimes found also in malaria of longer duration For instance Giordana (1938) in a series of radiological and clinical observations on 415 malaria patients repatriated from Italian East Africa on account of sickness found some form of cardiac disturbance in 60 and concluded that malaria was capable of producing circulatory and toxic changes in the myocardium the most important clinical signs of which were various degrees of cardiac dilatation

This author has also recently described right bundle branch block and T wave changes in malaria (1940) Sprague (1946) on the other hand found no cardiac abnormalities in a series of 50 cases of recurrent malaria studied electrocardiographically All except one of these cases were *P vivax* infections of up to 33 months duration the exception being a case of *P falciparum* malaria in which the infection had been acquired three months before examination In some cases the electrocardiograms were recorded after the chill during the febrile stage

Heart failure associated with extreme pathological changes in the cardiac muscle has been described especially by the older writers in the state of malarial cachexia As Sprague points out in such cases it is however impossible to distinguish between the effects of the malarial infection and those of the various nutritional deficiencies and other factors which accompany the condition

Kitchen (1941) says that cardiac failure accounts for most deaths in blackwater fever On the other hand Stephens after reviewing the literature up to 1937 concluded that renal failure caused death in half the fatal cases and heart failure in only a small proportion of the remainder In a series of fatal cases amongst British troops in West Africa in 1941–43 the findings were similar to those of Stephens Renal failure was the predominant feature in most cases and heart failure as distinct from generalized vascular collapse was seen in very few

In primate malaria the heart may also become involved in the tissue damage wrought by the disease but there is little clinical evidence

occasional ventricular and auricular premature beats low T waves associated with anaemia and sinus bradycardia and tachycardia In some cases functional systolic murmurs were detected Kean and Smith (1944) in a summary of the clinical findings in 100 fatal cases of *P falciparum* infection dying in the Canal Zone (Panama) between *19-5 and 1942 include cyanosis and gallop rhythm and a fast thready* pulse which might possibly have derived from cardiac involvement Vague bradycardia has been reported by Anderson (1927)

In more severe cases dilatation of the right side of the heart has been observed (Marchiafava and Bignami 1900) and many authors have attributed death to cardiac failure For instance Craig (1909) refers *to a case described by Ewing in which the clinical picture was primarily* one of cardiac failure associated pathologically with an overwhelming infection in which parasites were particularly concentrated in the vessels of the heart Seyfarth (19-6) concluded from analysis of the literature that cardiac and algid symptoms together could explain 14 per cent of all the deaths in malaria Kean and Smith (1944) however found in *their fatal cases of malignant tertian malaria little evidence that cardiac* failure *per se* was responsible for death Sprague s observations agree with those of Kean and Smith He found that malaria seldom caused death by direct myocardial involvement and that the heart was not found to be greatly affected in patients dying of other complications Kitchen (1941) included syncope and heart failure as a cause of death *in P falciparum* infections but did not give any estimate of the frequency of such deaths Other authors (e g Micheletti 1929 1930) refer to heart failure in acute malaria but do not distinguish between it as a purely terminal occurrence and as a predominant clinical state Substantial accounts of cardiac failure are given by Gaskell and Millar (1920) and Dudgeon and Clarke (1917) who describe it in malignant tertian cases in the 1914–18 war Dudgeon and Clarke investigated the cause of death in Serbians from malignant tertian malaria in Salonika during the 1914–18 war and noted that purely cardiac phenomena were frequent and were associated sometimes with collapse and sudden death They came to the conclusion that the cause of death in such cases was heart failure brought about by the intense degenerative changes demonstrated in the cardiac muscle at autopsy Gaskell and Millar in a similar series of cases also in Salonika described three modes of death in one of which the pathology centred round the heart They considered that as a result of repeated attacks of malaria and the added factors of fatigue and exposure cardiac failure developed in these cases and was the primary cause of death The predominant

found macroscopic changes in the heart in certain types of *P falciparum* infections There were no abnormal appearances in the heart after death from cerebral malaria but in the septicaemic and cardiac types of case the changes were often pronounced In the former in which cardiac failure was a terminal phenomenon the heart cavities were all greatly dilated and the muscle was soft pale and friable the vessels were grossly congested In the cardiac cases in which cardiac failure was the principal clinical feature the heart was again considerably dilated especially on the right side The muscle was pale flabby and very soft and the vessels were not greatly engorged Occasional small haemorrhages were found in association with the small veins Rigdon (1942) has reported changes similar to those described by Gaskell and Millar in their cardiac cases in a child which died from acute uncomplicated *P falciparum* malaria The heart muscle was pale and flabby and the cavities dilated Unlike most other authors however Rigdon reported that the blood found within the heart was fluid and contained no clot He regards this latter finding as significant since similar failure of the blood to clot is characteristic of shock (see Chapter XII) Nauck (1934) and Rigdon and Stratman-Thomas (1942) have described dilatation of the heart with flabby pale musculature in severe cases of acute *P knowlesi* malaria in monkeys

Histological changes

Parasites

The clot found in the heart cavities usually contains many parasitized red cells (Marchiafava and Bignami 1900 Craig 1909) Rigdon (1942) found parasites (1 per cent of which were segmenting forms) in the liquid blood which was present in the cavities of the heart in his case of *P falciparum* infection

Parasitized red cells and often free parasites can usually be found in most of the smaller vessels of the heart Sometimes they may be present in enormous numbers and may appear to block the vessels (Dudgeon and Clarke 1917 Craig 1909 Deaderick 1911) When present in large numbers they are usually accompanied by pigmented macrophages many containing phagocytosed erythrocytes both parasitized and unparasitized In general the heart vessels contain fewer parasites than those of the spleen and bone marrow Ewing s case (quoted by Craig 1909) in which the vessels of the heart muscle contained a greater proportion of parasites than the other organs of the body is exceptional two generations of parasites were observed

as a rule of cardiac failure except as a terminal event Rigdon and Stratman-Thomas (1942) found changes in the heart muscle in monkeys dying from acute *P knowlesi* infections associated in some animals with dilatation of the heart They suggested that myocardial failure might to some extent account for many of the lesions found in other organs e g the central necrosis in the liver It is more likely however that the lesions in the heart and other organs result from general circulatory failure rather than from primarily cardiac failure (see Chapter XII Rigdon 194- Moon 1938 Maegraith 1944 Kean and Taylor 1946)

It will be noted that opinion regarding the importance of cardiac involvement in malaria is divided On the one hand many authors consider cardiac failure to be the primary cause of death in a large proportion of their cases whereas on the other hand other workers have found it very infrequent except as a strictly terminal event This wide divergence of experience is due to some extent to confusion between cardiac failure *per se* and vascular collapse of the type of medical shock (Atchley 1930) in which the essential processes are quite different in that the heart is unable to maintain the circulation because of inadequate venous flow and not because of muscular failure Another explanation for the disagreement amongst observers may be that the more recent workers who have failed to find much evidence of cardiac failure have been dealing mainly with a selected population lying roughly with the young adult age group and under malarial control discipline It is also possible that strains of parasite vary in their effect on the myocardium most serious cardiac cases for instance have been reported from Europe whereas Sprague s cases (for example) were infected in the Pacific area

PATHOLOGICAL CHANGES

Macroscopic appearances

Marchiafava and Bignami (1900) reported flaccidity of the heart muscle and occasional subpericardial haemorrhages in acute malaria The chambers of the heart were dilated particularly those on the right side and often filled with coagula of corpuscles and fibrin Similar findings have been recorded by Craig (1909) who stated that there was little characteristic in the appearance of the heart except in very severe cases in which the muscle was anaemic pigmented and flabby Deaderick (1911) also noted a pale and flabby musculature but found few signs of degeneration in the muscle Gaskell and Millar (1920)

seems probable that the mononuclear cells described by Gaskell and Millar and others in the lumen of the vessels were circulating leucocytes and phagocytes

Thrombosis of the vessels of the heart is a rare phenomenon in malaria. Gaskell and Millar found no evidence of it. Other authors have reported that the vessels may be intensely congested and filled with red cells parasitized red cells and various kinds of white cells and phagocytes and malarial pigment but do not specifically mention thrombosis. Dudgeon and Clarke for instance also refer freely to blocking of the vessels of the heart with heavily infested red cells and suggest that such blocking may be of a temporary nature and quite distinct from either thrombosis or embolism

The earlier writers apparently considered that degenerative processes were seen only in the most severe cases of malaria (Craig 1909 Deaderick 1911) but in recent years it has been shown that degeneration and necrosis are common in the heart muscle even in cases which exhibit no clinical signs of cardiac involvement. Wenyon (1922) described degenerative changes in the muscle similar in many respects to those found in the toxaemia of diphtheria and toxic degeneration was demonstrated by Gaskell and Millar (1920) and Dudgeon and Clarke (1917). The former found that in cases of cerebral malaria there were often some degree of fatty degeneration of the muscle and what were described as globules of irregular size clustered especially thickly at the two poles of the nucleus. In one case of this kind there were finely scattered droplets of fat in some of the fibres which also showed some fragmentation. In the septicaemic type of infection characterized by a heavy parasitaemia the muscle fibres were fragmented and exhibited a pronounced degree of fatty degeneration the whole of the contractile substance being powdered over with fine globules of fat. Striation was poorly marked. In cardiac cases the lesions were of the same type as those in the septicaemic cases but not so extensive. Fat was again in evidence around the nucleus. Dudgeon and Clarke also noted the similarity between some of the changes in the heart muscle in acute malaria and those occurring in diphtheria. Fatty degeneration occurred in all of six cases in five of which the degenerative changes were diffuse as in diphtheria whereas in the sixth the distribution was irregular. The fat was present in the cells mainly in the form of fine droplets but also as medium sized and large masses. They believed that the fatty degenerative changes were probably related to the apparent blocking of adjacent capillaries and small blood vessels. Micheletti (1929 1930) reported myocardial

by Baker in enormous numbers in the blood of the heart in a fatal case of *benign tertian malaria (Marchiafava and Bignami 1900)* Gaskell and Millar (1920) found parasitized cells not very numerous in cerebral cases of malignant tertian malaria although the capillaries of the muscle usually contained some ring forms and a few rosettes In the septicaemic form of the disease parasites were extremely numerous and all stages of the life cycle from the ring form to the mature schizont were present Some parasites of the so-called filled in-ring type were found free in the muscle In the cardiac type of case intracorpuscular parasites were plentiful and in all stages of development In Rigdon s case the vessels of the myocardium were filled with parasitized red cells Similar findings have been reported in acute simian malaria (Taliaferro and Mulligan 1937 Rigdon and Stratman-Thomas 1942) Menon (1939) in *P knowlesi* infections in monkeys observed *parasites which like those described by* Gaskell and Millar appeared to lie free in the muscle cells On closer examination however these all proved to be within small vessels

Vascular and degenerative changes

The vessels of the cardiac muscle are sometimes found to be dilated congested and distended Occasionally small haemorrhages may be found in the muscle substance about the vessels especially the small veins Gaskell and Millar (1920) in their septicaemic cases described extreme congestion of all the large vessels and congestion of many but not all capillaries In some cases the latter were widely distended but were not filled with red cells In the lumen were parasites parasitized red cells free pigment and pigmented polymorphs together with cast off endothelial cells There was no marked perivascular cellular infiltration Deaderick (1911) states that the endothelium may be swollen pigmented and show fatty degeneration Gaskell and Millar describe finely granular pigment in the endothelial lining cells of the capillaries and arteries together with fatty degenerative changes They also describe degenerate endothelial cells in the vessels containing granules of pigment Few authors other than Gaskell and Millar have *however noted any phagocytosis by the endothelial lining* cells of the vessels although Taliaferro and Mulligan believe it may occur in exceptional circumstances and Merkel (1946) has recently reported it in *P falciparum* infections Micheletti (1929 1930) failed to find any evidence of engulfing of either parasites or pigment in a careful study of the heart in five cases of malignant tertian malaria He could also find no degenerative changes in the endothelium It

muscle as it is in the brain so that interference with blood flow in a given vessel is rapidly reflected in damage to the tissue it supplies Plugging occurs commonly in the vicinity of bifurcations of the small vessels (Merkel) a phenomenon also observed in the brain (Arieti 1946) Finally as in the brain the lesions tend to concentrate in regions where the anatomical arrangements of the blood supply are poorest in this case in the smaller branches of the vascular tree Merkel states that the lesions in the cardiac muscle in his cases resembled infarcts and that their distribution was patchy and not unlike that of emboli He and many others refer to thrombosis of the blood in the vessels in this connection but do not offer much firm evidence of its existence on any appreciable scale

The total occlusion or impedance of the blood flow to the heart muscle in an affected area leads to pathological changes in the tissue which are determined to some extent by the severity and duration of the malarial attack Two quite distinct types of degenerative change have been described in the heart muscle First there is the appearance of accumulations of small fat globules around the poles of the nuclei of the muscle cells and secondly the irregular development of diffuse fatty degenerative changes appearing as very fine globules of fat distributed through the cytoplasm of the cells

The globular degeneration resembles very closely that seen in the so-called brown atrophy of old age The granules are brownish yellow and do not take up ordinary fat stains It is doubtful whether they are functionally significant or primarily related to malaria since similar changes are frequently seen in the apparently otherwise normal heart muscle of Europeans who have lived for some time in the tropics

The second type of lesion is a fatty degeneration of the muscle cells associated with loss of striation and fragmentation and often with simple granular degeneration It resembles the toxic lesions of acute infections such as diphtheria and is probably a phanerotic change (Dible 1934) and does not represent any gross increase in the total fat of the organ There seems to be no available evidence on this point Such a change is probably reversible in its early stages

The degenerative changes in the muscle probably arise from deficient oxygen supply consequent on local circulatory failure aggravated by the malarial anaemia It is possible that the injury to the muscle tissue is facilitated by its considerable dependence for its function on carbohydrate metabolism which is very susceptible to oxygen lack The small changes in acid-base balance sometimes obtaining in the blood in malaria may also aggravate the injury to cardiac muscle

changes including fragmentation and necrosis of the muscle cells in two cases of malignant tertian malaria Lesions of the heart muscle resembling infarcts have been described recently by Merkel (1946) in two cases of *P falciparum* infection in both of which there was heavy parasitaemia The areas stained poorly with haemotoxylin and eosin and contained no fat The included muscle fibres had lost their striation and showed some hydropic degenerative change The capillaries were plugged with parasitized cells particularly at bifurcations where true thrombi appeared to have been formed It is not stated whether these thrombi contained fibrin Merkel believes that clinically the condition *of the heart in these cases is like any other form of coronary occlusion* Menon (1939) has reported atrophy and fatty degeneration in the muscle fibres in acute *P knowlesi* infections in *M mulatta*

Gaskell and Millar observed an increase in lymphocytes in some of their cases and interstitial myocardial infiltration with lymphocytes plasma cells and large macrophages has been recently reported by Spitz (1946) in cases of malignant tertian malaria which occurred during the 1939–45 war No other evidence of cellular infiltration or hyperplasia has been recorded in either human or monkey malaria

PATHOGENESIS

Heart failure occurring as a terminal event in acute malaria is seldom accompanied by specific pathological changes in the muscle other than those associated with general cardiovascular collapse In cases in which heart failure is a prominent feature of the disease and in long-standing or repeated infections lesions such as those described above are *commonly found namely plugging of the smaller vessels with packed* parasitized and non-parasitized erythrocytes and associated scattered areas of muscular degenerative changes The obstruction or retardation of the coronary blood flow which is brought about by the plugging of the capillaries probably depends on those factors which determine similar circulatory changes in other organs i e increase in endothelial permeability *leading to stasis agglutination of the type Knisely has* described mechanical obstruction due to changes in the endothelial lining cells of the capillaries and to the massed red cells phagocytes free pigment and parasites in the lumen

In many ways the vascular changes in the heart resemble those in the brain (Chapter IX) The endothelial cells lining the coronary vessels are relatively impermeable *so that increase of their permea*bility leads readily to stasis Collateral circulation is poor in the heart

CHAPTER XII

PATHOLOGICAL PROCESSES IN MALARIA

THE PARO YSM ANOXAEMIA Pulmo y f ct rs — F ct ffecting th eryth ocytes. THE RO E OF HAEMOZOIN CIRCULATORY PHENOMENA AND ANOXIA G l ci cul tory d s-tu bances — Local ci cul t y d sturbances — Th inut ti g f ctor FACTORS MODIFYING TISSUE RE PONSE Ho t pa as t ctions Nutrit l t t s of ho t N tur l immun ty A q ed immi nity Autoantigen- t body reactio s SYNTHESIS THE PATHOGENESIS OF MALARIA

THE PAROXYSM

ONCE synchronicity of maturation has been established and a pyrogenic density of parasites attained the appearance of the periodic febrile paroxysms corresponds closely to the time of sporulation This coincidence of maturation and paroxysm is easily demonstrated as a rule in *P vivax* or *P malariae* infections but may be difficult to show in *P falciparum* infections in which no high degree of synchronicity may be developed and in which mature forms of the parasite are seldom seen in the peripheral blood Nevertheless even in *P falciparum* malaria and in *P knowlesi* infections in rhesus monkeys in which there are no obvious paroxysms it is often possible to demonstrate some relation between the peak of maturation and changes in the blood usually associated with the paroxysm (Zwemer *et al* 1940)

It is therefore assumed that there is some causal relationship between the maturation of the parasite and the development of the paroxysm although it is not clear what this relation is

When merozoites are liberated into the plasma at the disruption of the erythrocytes containing the ripe schizonts there are simultaneously released red cell debris the remains of the cytoplasm of the schizont not included in the merozoites malarial pigment and probably various soluble chemical constituents of the red cell and parasite The symptoms and physical signs which accompany this process include a rapid rise of bodily temperature frequently accompanied by rigor and certain changes in the chemical constituents of the blood including a rise in plasma potassium a fall in sodium concentration and a rise and fall in blood sugar cholesterol and phospholipoids

These phenomena are essentially the same as those produced by many non-specific pyrogenic agents e g typhoid vaccine Altschule

which is unusually sensitive to accumulation of acid metabolites such as lactic acid

Brown and Loevenhart (1913) investigated the action of (alkaline) haematin (which Brown and subsequent workers have identified with haemozoin) on the circulation of dogs and cats and reported that it caused bradycardia dilatation of the coronary vessels constriction of the superficial vessels of the legs and dilatation of the vessels of the gut They considered that haematin which as Brown (1912) had previously shown in rabbits could give rise to phenomena resembling those of the paroxysm was thus a possible toxic factor in the production of cardiac changes in malaria Anderson and Morrison (1942) have however recently shown that it is unlikely that malarial pigment has any effect on the coronary vessels since haematin is not liberated from the parasites in a soluble form and does not appear in the plasma in solution

change in the oxygen concentration of arterial blood in either chill or flush but that of the venous blood fell in the chill and rose in the flush until it approached that of arterial blood There was no change in blood plasma volume or haematocrit values Examination of the nail-bed capillaries showed that these vessels constricted sharply in the chill and remained constricted at the onset of the flush When the latter became established the capillaries opened widely and contained rapidly moving blood Defervescence was associated with a gradual return to normal

These findings were interpreted as follows The onset is marked by a generalized vasoconstriction which includes arterioles and accounts for the rapid rise in blood pressure Since visible veins were also constricted in this phase and no change in circulatory blood volume could be demonstrated they concluded that there must be compensatory vasodilatation in some areas The latter did not include the kidney or the brain in both of which a decrease in blood flow had been demonstrated in the chill stage of induced fever The vasoconstriction probably accounted for the decreased circulation time In the flush there was capillary venous and arteriolar dilatation and an associated fall in blood pressure The blood volume was again unchanged indicating vascular constriction in some regions of the body The renal flow in this stage sometimes increased The intense oxygenation of the venous blood suggested the opening up of arterio venous shunts The acceleration of the circulation time and the increase in cardiac output were greater than would be expected in view of the increased metabolism of the fever Altschule suggests that there was therefore an increased venous return to the heart

A rise in respiratory rate in the cold and hot stages of the paroxysm occurs in malaria and a similar change has frequently been observed in artificial fever Altschule found this associated with a fall of alveolar air carbon dioxide most pronounced in the chill and with changes in the plasma in the direction of alkalosis He suggests that hyperventilation which is probably responsible for the alkalosis may be initiated by the rise in the temperature of the blood circulating through the brain

The clinical phenomena of the paroxysm can thus be very closely reproduced by injection of pyrogenic agents in no way associated with malaria They are probably therefore non specific It is commonly believed that their immediate production must depend on some product of sporulation, liberated into the plasma by the disintegration of

et al (1945) found that injection of typhoid vaccine after a short prodromal stage was followed by a chill in which there was increasing pallor some cyanosis and hyperventilation nausea and vomiting The patient complained of cold and shivered but the rectal temperature rose steadily The cold stage lasted one to one and a half hours and was followed by a brief hot stage which was terminated by drenching sweat In the hot stage which Altschule called the flush the cutaneous vessels dilated and the rectal temperature remained high The patient complained of feeling hot and had a severe pounding headache The flush lasted about an hour The skin during the chill was pale and cold except for some cyanosis of the extremities In the hot stage it was flushed and warm

Altschule found a rise in both systolic and diastolic blood pressures in the early stages of the chill followed by a progressive fall of both pressures in the flush sometimes starting towards the end of the cold stage The diastolic pressure fell relatively faster than the systolic so that the pulse pressure increased during the flush and in the early stages of defervescence

The similarity between Altschule s findings and the phenomena of the malarial paroxysm is striking The skin in the cold stage of the paroxysm is pale and cold due to vasoconstriction and in the hot stage flushed due to vasodilatation The blood pressure changes are almost identical with those observed in the vaccine reaction For instance in benign tertian malaria Mikeladse (19_3) observed a rise of systolic blood pressure in the cold stage of _0–30 mm Hg above the pressure in the apyrexial period This high pressure was maintained for the first hour of the hot stage and then fell rapidly finally reaching a level below that of the apyrexial period about the end of the sweating stage There was a rise in diastolic pressure in the cold stage and a considerable fall in the hot stage the fall in this case being relatively greater than that of the systolic pressure so that the pulse pressure was increased This fall in diastolic pressure continued throughout the sweating stage after which there was a gradual return to normal In all there was a variation of 30–50 mm Hg and 15–20 mm Hg in systolic and diastolic pressures respectively between the beginning of the rigor and the end of the paroxysm Mikeladse s findings have recently been confirmed by Flost (1944)

Altschule found the pulse rate was roughly proportional to the temperature The cardiac output increased in half his cases during the chill and in all during the flush The arm-to-tongue circulation time was slowed in the chill and accelerated in the fever There was little

and found it lasted about 45-60 minutes It was terminated by dilatation of the skin vessels the ears becoming hot and flushed This stage was considered to be the equivalent of the hot stage of malaria During the chill stage there was at first little change of temperature but after a short time it rose sharply and reached a maximum before the stage of vascular dilatation in which it fell rapidly to normal

Brown and Loevenhart concluded from their experiments that haematin (haemozoin) was a potentially toxic substance They discussed the possibility of its liberation in soluble form in the infected subject and believed that this was unnecessary since colloidal injections of haematin gave rise to the same syndromes in animals as solutions

Duesberg (1934) found that intravenous injection of crude ferrihaemate solutions prepared from once crystallized haematin caused symptoms slightly resembling those of the paroxysm including chills and fever but he could not reproduce these effects if more highly purified pigments were used

Fairley and Bromfield (1934) and Fairley (1938 1939) in experiments originating in a series of blackwater fever cases showed that free ferrihaemate added to blood rapidly unites with crystalbumin to form apparently inert methaemalbumin and suggested that this was the process by which the body normally dealt with haematin If this is the case in intravascular haemolysis such as occurs in malaria it is unlikely that haematin in itself can be a very toxic factor Anderson and Morrison (194_ a and b) however found that disodium ferrihaemate injected in big doses into dogs by intraperitoneal intravenous or subcutaneous routes persisted in the blood for up to two days and was not excreted in the urine There was no accompanying change in serum bilirubin but the excretion of porphyrin in the urine was increased (Fairley believes methaemalbumin may be excreted eventually as a porphyrin) In these animals there was no elevation of temperature but extensive damage was produced in certain organs

Five monkeys were later given disodium ferrihaemate intravenously and similar changes were found to those in the dogs Sixteen animals were given *P knowlesi* infections in some of which the strain was attenuated and there were two normal controls After the injections there was a stimulation of respiration and increase in heart rate The surface vessels constricted and the face became blanched and cyanotic There was no constant change in temperature which was however sometimes raised In the injected animals the pigment could be detected in the plasma but it was never found in solution in the infected

infected cells There is no evidence implicating the merozoites themselves it is clearly not necessary to have living cells present to initiate the paroxysm

Many authors have held that a specific soluble product of the parasite or a by-product of its metabolism was responsible not only for the paroxysm but for the whole clinical picture of malaria (Meleney 1941 Viswanathan 1944) Wells (1926) stated for instance that the plasmodia undoubtedly produced toxic substances which did not diffuse from the red cells but were liberated only at the maturation of the parasite The nature of these toxins was unknown to Wells and is still unknown in spite of a fairly vigorous search Various attempts have been made to identify substances responsible for the paroxysm and other phenomena associated with malaria For instance it has been claimed that extracts of parasites are strongly haemolytic and that malarial serum haemolyses normal red cells These claims have never been verified Lintwarcff (1937) attempted to show that there was a specific element in malarial plasmodia capable of combining with haemoglobin to form a compound acting directly on the epithelial cells of the kidney tubules but was unsuccessful Other attempts to demonstrate specific toxic substances associated with the parasite have also failed On the other hand both the malarial pigment and the protein substances presumably liberated at the time of the disintegration of infected cells have been implicated in the pathogenesis of the paroxysm and are still believed by some to be involved in it

Brown (1912) and Brown and Loevenhart (1913) considered haemozoin to be a factor of primary importance They injected a solution of (alkaline) haematin (which they previously and correctly identified with malarial pigment) intravenously into rabbits and obtained chills similar to those of the malarial paroxysm Solutions of sodium hydroxide were used as controls The animals all responded to dosage by a sharp rise in body temperature which reached a peak about an hour after the injection This was followed by a short rapid fall of temperature and a more gradual fall to normal over the course of some hours The extent of rise of temperature was roughly proportional to the dose More severe effects were produced by dividing a given dose into two or three fractions injected at intervals of 15–30 hours (corresponding roughly to the release of pigment at sporulation) The clinical appearance of the animals was characteristic There was a short preliminary period following injection in which the ear vessels constricted and the skin became cold and cyanotic In this state there was shivering and tremors and sometimes shaking Brown called this the chill stage

the similarity between the changes they observed in blood sugar during the paroxysm and those reported in protein and anaphylactic shock (Kuriyama 1917 Moon 1938) and suggested that some form of shock may be concerned in the production of the paroxysm Velick and Scudder (1940) also noted the similarity between the symptoms of certain forms of avian malaria and those of anaphylactic shock They found that there was a rise in plasma potassium corresponding to a broad peak of sporulation in *P cathemerium* infections of canaries and suggested that the excess potassium came from the destruction of the parasitized erythrocytes Flosi (1944) and Zwemer *et al* (1940) observed similar increases of potassium in human and monkey malaria respectively in the former case associated particularly with the beginning of the paroxysm The excess of potassium was more than could be accounted for by red cell destruction and on the analogy of similar increases in anaphylactic and other forms of shock (Moon 1938) it was suggested that the paroxysm might result from an anaphylactoid reaction possibly brought about by sensitization of the tissues to some products of sporulation It has been found however that high concentrations of potassium occur in other conditions which may equally well develop in malaria such as adrenal cortical insufficiency and certain states of anoxia in which there is an initial stimulation of the adrenal medulla with consequent liberation of adrenalin and mobilization of potassium from the tissue cells The release of adrenalin would also mobilize glucose and account for the hyperglycaemia reported by Sinton and Kehar and others

Zwemer Sims and Coggeshall (1940) point out that high plasma potassium concentrations are in themselves toxic and may account to some extent for the phenomena of the paroxysm possibly owing to their effect on the adrenal cortex Similar arguments have been produced in support of the contention that high potassium levels cause the symptoms of certain forms of shock but Manery and Solandt (1941) have shown experimentally that the toxic effects of potassium are manifested only at much higher concentrations than those obtaining in shock Since the latter are of the same order as those seen in malaria the observations of Manery and Solandt apply equally to malaria Nevertheless the raised potassium concentrations during the paroxysm in man may account for some of the clinical reaction since it has been found that the rigor may be abolished by the intravenous injection of a solution of calcium chloride (Beeson and Hoagland 1940) The effect of this calcium salt is not clear but it is possible that it acts by readjusting the potassium calcium balance in the blood which is upset by the

monkeys in one case a pigment similar to Fairley s methaemalbumin was isolated

Morrison and Anderson point out that the invaded red cells in the *parasitized animals are dehaemoglobinized by the parasites and the* haemozoin formed is contained as granules within the parasite and not liberated as their experiments showed as a solution in significant quantities in the plasma They therefore consider that there is no direct causal relationship between the parasite pigment and the paroxysmal symptoms The significance of pigment in the pathogenesis of tissue changes will be discussed elsewhere

The rupture of parasitized red cells does not discharge significant amounts of haemoglobin into the plasma since by the time of maturation the bulk of the pigment has been converted into haemozoin Moreover the lysis of unparasitized cells during the paroxysm gives rise to no more than traces of haemoglobin in the plasma (Fairley and Bromfield 1933) Since large amounts of haemoglobin may be injected without injury into normal animals this pigment cannot therefore be regarded as the initiating factor in the production of the paroxysm

During the clinical activity of malaria the total protein of the plasma is usually reduced due to a considerable fall in the albumin fraction which is compensated for to some extent by a rise in the globulins particularly the euglobulin fraction Peterson (1926) observed a fall in total plasma proteins at the beginning of the paroxysm followed by a return towards normal in the apyrexial period A similar fall was noted by Radosavljevic and Ristic (1926) at the height of the paroxysm and by Wiechmann and Horster (1926) at the onset of the fever These changes in plasma protein content are principally brought about by disturbance of synthesis in the liver and are of such magnitude that any quantitative change occurring during the paroxysm as a result of liberation of protein from the disrupted erythrocytes would be very small No such increase in any fraction of protein during the paroxysm has apparently so far been detected It is therefore uncertain whether protein in any appreciable quantity is liberated at sporulation Any reaction due to such release is thus probably of an indirect nature and difficult to determine Some authors however have drawn attention *to the close similarity between the phenomena of the paroxysm and* those of anaphylactic shock Abrami and Senevet (1919) for instance have suggested that the paroxysm is an anaphylactic reaction resulting from the sudden release of the protein of the parasites or cell debris into the plasma at sporulation Sinton and Kehar (1931) pointed out

the similarity between the changes they observed in blood sugar during the paroxysm and those reported in protein and anaphylactic shock (Kuriyama 1917 Moon 1938) and suggested that some form of shock may be concerned in the production of the paroxysm. Velick and Scudder (1940) also noted the similarity between the symptoms of certain forms of avian malaria and those of anaphylactic shock They found that there was a rise in plasma potassium corresponding to a broad peak of sporulation in *P cathemerium* infections of canaries and suggested that the excess potassium came from the destruction of the parasitized erythrocytes Flosi (1944) and Zwemer *et al* (1940) observed similar increases of potassium in human and monkey malaria respectively in the former case associated particularly with the beginning of the paroxysm The excess of potassium was more than could be accounted for by red cell destruction and on the analogy of similar increases in anaphylactic and other forms of shock (Moon 1938) it was suggested that the paroxysm might result from an anaphylactoid reaction possibly brought about by sensitization of the tissues to some products of sporulation It has been found however that high concentrations of potassium occur in other conditions which may equally well develop in malaria such as adrenal cortical insufficiency and certain states of anoxia in which there is an initial stimulation of the adrenal medulla with consequent liberation of adrenalin and mobilization of potassium from the tissue cells The release of adrenalin would also mobilize glucose and account for the hyperglycaemia reported by Sinton and Kehar and others

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rise in potassium since the calcium concentration remains practically unchanged in malaria

An increase in blood cholesterol concentration has been observed by Kehar (1937) during the paroxysm in *P vivax* and *P falciparum* infections This increase occurs early in the cold stage and may be manifest before the rigor A maximum concentration is reached within three hours of the start of the paroxysm and is followed by a sharp fall to subnormal values Kehar suggested that this rise of blood cholesterol in the paroxysm may be associated with the general acceleration of metabolism especially that of fat due to the fever A similar hypercholesterolaemia has been observed in anaphylactoid shock in animals and as part of a general lipaemia after exposure to anaemic anoxia following haemorrhage or destruction of erythrocytes with phenylhydrazine and anoxic anoxia following exposure to atmospheric oxygen deficiency

The genesis of the paroxysm

There is an obvious relation between the maturation of the schizonts and the development of the clinical phenomena of the paroxysm but the factor initiating the latter has not yet been determined It is clear however that the febrile paroxysm is a non-specific reaction on the part of the host and is similar to the bodily reaction to other acute pyrogenic agents

The mechanism which promotes the rise of temperature is unknown but is probably similar to that which gives rise to fever following injection of typhoid vaccine In the latter case the fever may be reduced or prevented by destruction of the hypothalanus (Ranson *et al* 1939) so that stimulation of this centre may be responsible for the febrile response

A factor which influences the rise of temperature and may in part be responsible for it is the increased metabolic activity associated with the combination of violent shivering and interference with the ordinary heat lo s mechanisms of the skin arising from the intense peripheral vasoconstriction Du Bois (19__) found that in a normal subject voluntarily simulating the muscular activity of a rigor for some time the metabolic rate rose about _00 per cent although the body temperature changed only slightly In a patient infected with *P vivax* and undergoing a rigor the metabolic rate was raised to the same degree but the heat loss was greatly diminished because of peripheral vascular constriction so that the body temperature rose sharply In the cold stage of the paroxysm therefore the patient is producing

more heat than normal and losing less The temperature of the blood consequently rises whilst that of the skin falls due to vasoconstriction

In paroxysms in which rigor is not evident muscular activity can have no part in the elevation of body temperature Some other mechanism must therefore be responsible for the fever and it is probable on the analogy of experimental results in artificially induced fever that the hypothalamic centres are directly involved presumably as the result of the release into the circulation of some diffusable substance liberated at the time of sporulation

When the patient passes into the hot stage the skin vessels dilate and heat loss from the skin is accelerated This vasomotor phenomenon is also probably hypothalamic in origin and is the response to the rising blood temperature *per se* During the fever the metabolic rate is raised but not enough to maintain the temperature at the prevailing high level (Best and Taylor 1943) In this case again the temperature remains elevated because heat loss is inadequate to compensate for heat production and the temperature remains high With the appearance of sweating heat is lost rapidly and the body temperature falls to normal and often below The temporary inhibition of sweating during the hot stage and its sudden appearance and vigorous activity in the last stages of the reaction have not been explained but it is possible that in malaria as in other febrile reactions it may correspond to the withdrawal of inhibition of the sweating centres following the disappearance of the causative factor from the circulation

The subjective feeling of chill is probably due to the constriction of the skin vessels in the region of the cutaneous heat receptors the sudden reduction of local circulation having much the same effect as a plunge into cold water Barbour (19_1) however has suggested that the factor initiating the cold stage (in the case of bacterial toxins) may act by allowing some escape of fluid from the vessels resulting in a reduction of circulating blood volume which is compensated for by peripheral vasoconstriction Vasoconstriction associated with loss of blood volume takes place in the vascular collapse of pernicious malaria but there is no evidence to suggest that any appreciable general change in circulating volume occurs in the ordinary case According to Altschule *et al* (1945) in the response to injection of typhoid vaccine even in apparently shocked subjects there is no change in blood volume either in the cold stage or subsequently This indicates that accompanying the peripheral constriction of the chill and dilatation of the hot stage there may be respectively dilatation and constriction

of vessels elsewhere Altschule believes that in the chill the vascular dilatation is chiefly centred in the lung

The failure of all attempts to isolate specific parasitic toxins and the obvious *relationship between the appearance of the paroxysm* and the final stage of schizogony have led many authors to the view that some kind of anaphylactoid shock can best account for the reaction and the raised glucose cholesterol and protein blood concentrations observed during the paroxysm have all been stressed in support of this hypothesis It is difficult however to conceive of an anaphylactic reaction which can occur with undiminished severity every two or three days for weeks or even months The clinical picture could equally well result from a reaction to a so far unidentified pyrogenic agent (which is certainly not either haemozoin or haemoglobin but which might be a metabolite of the dividing parasite or a by-product of schizogony) appearing periodically at the completion of each asexual cycle and affecting the thermogenic hypothalamic centres thereby initiating a series of vasomotor disturbances causing decrease in heat loss and increase in blood temperature and other physiological responses including hyperventilation

Considering the close similarity between the paroxysm of malaria and the febrile response to ordinary pyrogenic agents such as typhoid vaccine there seems no need to postulate any form of anaphylactoid reaction All the biochemical phenomena of the paroxysm that have been observed are non-specific and may be accounted for by humoral mechanisms The circulation of excess adrenalin for example would adequately explain amongst other things the high blood potassium and glucose concentrations It is possible therefore that the initiating agent may act by stimulating the adrenal gland medulla directly There is no evidence of such direct stimulation however and it may equally well be that the humoral mechanisms are set in motion by the rapid development of a state of anoxia in the adrenal brought about by local vasoconstriction initiated for instance by stimulation of the hypothalamus Hyperglycaemia hyperkalaemia and hypercholesterolaemia are all phenomena which occur in anoxia and there is some evidence to suggest they derive from activity of the adrenal since they do not appear under anoxic conditions in adrenalectomized animals A local state of anoxia may arise following local vasoconstriction in the organ concerned and that this is possible in the paroxysm is shown by the blood pressure changes which indicate an extensive arteriolar constriction in the cold stage In induced febrile reactions the blood flow in certain organs e g the brain and the kidney has been

found to be reduced as well as that of the skin (Chasis Ranges Goldring and Smith 1938 Himwich Bowman Goldfarb and Fazekas 1939) but there appears to be no information regarding the adrenal

Recapitulation

The clinical features of the malarial paroxysm are essentially those of a reaction to a non-specific pyrogenic agent such as typhoid vaccine The factor initiating this reaction in malaria has not yet been identified There is little evidence to support the view that a specific malarial toxin or metabolite is involved Haemoglobin and haemozoin are not responsible The hypothesis that an anaphylactoid reaction is involved is not confirmed most of the humoral phenomena upon which this theory is based can be explained on the grounds of the production of increased quantities of circulating hormones such as adrenalin It is suggested that the latter may result from acute anoxia of the adrenals due to local vasoconstriction this vasoconstriction resulting from hypothalamic stimulation by some non specific pyrogenic agent liberated at sporulation of the parasite

ANOXAEMIA

Pulmonary factors

Changes in pulmonary ventilation occur in all forms of severe malaria and may limit the oxygenation of the blood and so contribute towards the prevailing anoxaemia Clinical signs of pulmonary involvement are also not uncommon especially in malignant tertian (Falconer 1919) Spitz (1946) for example found pulmonary oedema in 50 consecutive cases sometimes accompanied by bronchopneumonia or interstitial pneumonitis The basic tissue changes in these cases were intense dilatation and hyperaemia of the septal capillaries which were usually filled with (but not obstructed by) parasitized erythrocytes and occasional small haemorrhages which resembled those in the brain in that the constituent cells were non-parasitized No thrombosis was observed but it is evident that the blood flow through the dilated engorged vessels must have been retarded and the oxygenation of the circulating erythrocytes correspondingly reduced

Factors affecting the erythrocytes

The destruction of parasitized erythrocytes during sporulation and the lytic and phagocytic processes which affect parasitized and unparasitized cells alike bring about a notable reduction in red cell numbers

This if not compensated for adequately by medullary or extra medullary haemopoiesis leads to anaemia the degree of which is dependent to some extent on the species of invading plasmodium (Chapter III) The oxygen carrying capacity of the blood is reduced in proportion to the anaemia and this in itself as has been shown in other anaemias will ultimately affect the metabolism of the tissues Anoxaemia resulting from erythrocyte destruction has in fact been considered the immediate cause of death in intensive *P relictum* infections in pigeons (Hill 1942) The anoxaemia produced by the development of anaemia however is exaggerated in malaria because of the invasion of the extant erythrocytes Christophers and Fulton (1938) pointed out that the pallor of the infected cells indicated that the parasite in some way removed the haemoglobin from the cell The demonstration of the formation of haemozoin by the parasite by Ghosh and Sinton (1934) and the more recent work on the intracellular production of haematin strongly supports this view (Morrison and Anderson 1942) Christophers and Fulton have succeeded in showing that in *P knowlesi* infections in rhesus monkeys progressive loss of haemoglobin does take place in the infected cells They were not able to demonstrate this phenomenon colorimetrically but by using a method based on the estimation of the oxygen capacity of freshly defibrinated infected blood they detected a definite loss of haemo globin in the parasitized blood They also observed that of two samples of blood examined the one containing the greater proportion of parasites to total cells showed the greater loss in haemoglobin indicating that the loss in haemoglobin was related to the presence of the parasites

The fate of the haemoglobin used up by the parasite in its metabolism is discussed elsewhere (Chapter II) It is important to realize that these processes are apparently entirely intracellular and do not lead to the presence of blood pigments in the plasma or tissues

The loss of effective haemoglobin from the invaded red cells must further reduce the oxygen capacity of the blood but another factor of even greater importance intensifies this effect Christophers and Fulton have shown that the parasite can readily take its oxygen from the oxyhaemoglobin of the cell They suggest that it normally acquires oxygen in this manner since they found the oxygen uptake of the parasite was extremely sensitive to the inhibitory action of drugs which affected the red cell If the contention of these authors is correct the invaded cells must be reckoned as poor carriers of oxygen to the host s tissues since not only is the haemoglobin content of the ery-

throcyte reduced by the invasion but the parasite is constantly competing for the oxygen combined with the remaining pigment It is possible also that the presence of the parasite perhaps through the medium of some *diffusable product may affect the utilization of* available oxygen by tissue cells and influence the dissociation of oxyhaemoglobin in such a way that oxygen is neither readily received nor easily given up The experimental results of Christophers and Fulton could conceivably be interpreted in this manner particularly if the haemoglobin of the non parasitized cells were also involved The tissue alkalosis produced in certain cases in which there is anoxia associated with hyperventilation may also have an adverse effect on the dissociation

Where the infection of the red cells is heavy e g in *P falciparum* malaria the factors mentioned above probably play an important part in the pathogenesis of the disease in general and particularly in the production of local lesions in organs like the brain in which stasis of capillary vessels also occurs Here the smaller vessels become loaded with temporarily static erythrocytes including parasitized cells which are both short of haemoglobin pigment and deficient in oxyhaemoglobin

The efficiency of the erythrocytes as oxygen carriers is also reduced in malaria by processes which lead to their agglomeration The *most important phenomenon* of this kind is the intravascular agglutination described by Knisely and his colleagues which leads not only to ineffective oxygenation of the agglutinated cells but also to interference with the blood flow through the small blood vessels Intravascular agglutination has been studied in transilluminated living tissue by Knisely in both human and monkey malaria and by Lack (1945) in avian malaria In normal control animals Knisely (1945) found circulating red cells were not agglutinated the white cells did not stick to the endothelium and the flow through the vessels was streamlined In monkeys in the last stages of *P knowlesi* infections the picture changed completely A thick glassy precipitate formed between and around the erythrocytes binding them together into wads and masses —not rouleaux This precipitate formed simultaneously throughout the body of the affected animal the process taking only a few minutes Small clumps of red cells were thus formed in the circulation and tended to coalesce forming a sort of loose sludge which was passed with difficulty through the smaller vessels and impeded the blood flow the streamlined appearance of which was disturbed At this stage the endothelial lining of the vessels appeared sticky to leucocytes but not to the affected red cells The latter were however sticky to phago-

cytes which avidly engulfed them A very similar picture has been described by Lack in birds infected with *P cathemerium* As the parasite count rose there developed a transient stickiness of leucocytes to the endothelium This was followed by adherence of the white cells to the endothelium and the formation of clumps of red cells the clumping progressing steadily over the course of 24-48 hours Lack stated that the clumps were originally formed only by the parasitized cells but that later all cells were affected The final state in the bird was a paste-like blood flow

Knisely considered the crystalline deposit to be fibrin It was at first thought to be related to some kind of immune response on the part of the host but recently Knisely (1943) has reported that similar clumping may be seen in man in acute alcoholism and a variety of other human diseases and has reported it in experimentally induced traumatic shock (1945) In the latter experiments he obtained evidence to the effect that striated and smooth muscle when crushed release substances which can diffuse through the vessel walls and bring about a sludging effect on the contained red cells

It seems therefore that the autoagglutination observed by Knisely and his colleagues in malaria is not a phenomenon which is specific to the infection It occurs extensively only very late in the disease when there is considerable parasitaemia Its influence on the pathogenesis of malaria is not clear since its non-specificity and late appearance suggest that it may itself be the product of the final stages of anoxia in which changes somewhat similar have been described by earlier workers For instance Liebesny (1921 1922) in observations on the effect of atmospheric anoxia on the flow through small vessels found that the appearance of the capillary circulation was altered so that the flow was no longer rapid and homogeneous but beaded and irregular as a result of what appeared to be sedimentation or agglutination Schneider and Truesdell (19-4) found similar changes in extreme anoxia the red cells sometimes showing clumping

Whether it is specific in any way to the malarial infection or not agglutination of red cells with the formation of sludge if formed early enough in the course of the infection (as is suggested by Lack's observation in birds) would have a profound effect on the circulation through the smaller vessels leading to obstruction and aggravation of the prevailing state of tissue anoxia

There is considerable doubt concerning the relation between this agglutination of red cells and the appearance of true thrombosis Knisely claims that the sludge slows the capillary circulation to the

point of producing stagnant anoxia and that this in turn affects the permeability of the vessel wall and allows the escape of fluid to the tissues ultimately leaving the vessel plugged with stranded masses of precipitate-coated tightly agglutinated cells If this were commonly the case the fibrin should be obvious in many of the apparently occluded vessels seen in fatal malaria It is in fact seldom seen This may be because the damage to the finer circulation is already effected by stasis before the sludge is formed or because some process of fibrinolysis removes the fibrin It may be that the vessel obstructed by sludge immediately goes into a state of stasis so that the fibrin precipitate (if it is fibrin) is insignificant in relation to the obstruction There is room for a great deal of experimental work on this problem

THE ROLE OF HAEMOZOIN

Brown and his colleagues (1911 1912 1913) identified haemozoin as haematin (subsequently confirmed by Ghosh and Sinton 1934 Devine and Fulton 1941 Anderson and Morrison 1942) and injected alkaline solutions of haematin extracted from rabbit dog and ox blood into rabbits in quantities corresponding to those which might possibly be liberated from the parasites during sporulation in man They obtained results described above in the discussion on the paroxysm which led them to conclude that the malarial pigment was at least a potentially toxic substance Subsequently experiments by Duesberg however showed that carefully purified haematin had few harmful effects in man and as explained above Morrison and Anderson (1942) have recently come to the conclusion as a result of experiments in dogs and monkeys that there is no direct causal relation between the symptoms of malaria and the parasitic pigment

The lesions in the organs of dogs and monkeys resulting from the injection of soluble haematin were found by Anderson Morrison and Williams (1942) to be a combination of accumulation of pigment in the reticuloendothelial system vascular abnormalities and renal changes There was generalized vasodilatation with haemorrhages of various sizes in many tissues Thrombosis and infarction were present in some organs the thrombosis sometimes consisting mainly of pigment although fibrin was present in most of them The renal lesions were mostly vascular in origin but there was marked degeneration of the convoluted tubules Casts were plentiful but not pigmented The pigment was stored in the reticuloendothelial system and in agreement with Duesberg and others they observed no increase in the

plasma bilirubin content following the injection They concluded that the pigment was harmless when held in the tissues but when free in the circulation it produced intense vascular reactions which gave rise to congestion haemorrhages and thromboses The renal changes arose from similar vascular reactions

In a subsequent paper reviewing the lesions found in simian malaria Anderson and Morrison (1942) showed that there were significant differences between the lesions of simian malaria and those produced by the injection of pigment For instance the distribution of pigment was different Relatively little of the injected pigment was found in the spleen and bone marrow whereas in animals infected with malaria the spleen was chiefly involved Moreover the symptoms in the infected monkeys did not appear until anaemia had become severe They concluded that the most probable explanation of the lesions in simian malaria was that they arose from anoxaemia due to vascular occlusion associated with severe anaemia They did not consider that haemozoin was implicated since it was apparently not liberated in a soluble form from the parasites This view is now generally accepted

CIRCULATORY PHENOMENA AND ANOXIA

Changes in the circulation of both general and local nature are supremely important factors in the pathogenesis of malaria To a certain extent the general circulatory phenomena affect local tissues but in many organs specific local vascular changes are brought into play during the progress of the disease It is necessary therefore to define the general and local changes separately Many of the latter have been discussed elsewhere in the chapters dealing with the particular organs concerned

General circulatory disturbances

In the paroxysm during the cold stage there is peripheral vasoconstriction an abrupt rise in systolic blood pressure and a smaller rise in diastolic As the chill subsides and the hot stage develops vasoconstriction gives place to peripheral dilatation accompanied by a fall in blood pressure more pronounced in the diastolic than the systolic Conditions are gradually restored to normal during defervescence During the cold stage the metabolic rate is raised as much as 200 per cent but the heat loss is prevented by the vasoconstriction so that the body temperature rises rapidly In the hot stage heat loss

by the skin is only partly restored and the circulatory effects of fever appear The condition of the circulation during the hot stage is thus the physiological reaction to raised metabolism and excessive bodily heat The vasoconstriction of the cold stage is more difficult to understand On the analogy of paroxysms produced artificially by the injection of pyrogenic vaccines and of experimental work on heat control in animals it seems likely as argued above that the changes in malaria may be initiated by some factor acting directly on the hypothalamic centres

In the normal course of events the hot stage of malaria lasts only a few hours and the temperature becomes normal and remains so until the next paroxysm Sometimes especially in *P falciparum* infections the temperature remains elevated so that the metabolic rate is raised and the cardiac output stays at a high level placing an unrelieved strain on the heart As explained elsewhere (Chapter XI) this rarely goes on to heart failure although occasionally changes in the heart muscle itself cause its failure as a pump

Much more frequently some degree of vascular collapse develops which in the extreme form is indistinguishable from medical shock (Atchley 1930) Vascular crises are seldom well developed in the paroxysm but may occasionally occur and have been recorded in artificially induced fevers associated with rigors In the latter case there are important differences between the condition which develops and the state of shock since according to Altschule (1945) there is no change in blood volume Nevertheless the patient is pale the skin cold and sometimes sweaty and there is a fall of arterial blood pressure and a low cardiac output This state is transitory and disappears at the commencement of the hot stage

In some cases a persistent rise in temperature may precipitate a vascular crisis especially if there is also uncompensated loss of fluid In similar circumstances in induced fever Bazett (1931) has shown that the blood volume may be diminished and the venous return to the heart reduced leading finally to circulatory failure Instances of such failure have been recorded by Kopp and Solomon (1937) who reported the development of medical shock in eight cases of therapeutic hyperpyrexia induced by hot moist air In these cases two of which were fatal impending shock was demonstrated by increased pulse rate pallor and cyanosis of the skin and a continued rise of temperature associated with a falling blood pressure Signs of pulmonary oedema appeared in three patients Hartman (1937 1938) described three similar fatal cases developing during induced pyrexia

and many others have been reported after exposure to high external environmental temperatures under natural conditions

The vascular disturbances of malaria are however seldom associated with hyperpyrexia and profound circulatory disturbances are more commonly found in other forms of pernicious malaria particularly the algid type The appearance and physiological state of the patient in algid malaria is that of severe shock The skin is dehydrated moist and cool the lips and extremities cyanotic The pulse is rapid thin and easily compressed the respirations superficial and irregular The temperature is sometimes elevated but may be subnormal The blood pressure is low and there is evidence of haemoconcentration Paisseau and Lemaire considered that this syndrome resulted from derangements of the adrenal glands but the evidence of this is often lacking in individual cases According to Kean and Taylor (1946) it was Gage (19-6) who first suggested that the condition was probably one of shock Atchley (1930) reported a similar state in numerous other diseases including typhoid and influenza and grouped them as medical shock which Fishberg (1940) later described as fundamentally similar to traumatic shock but differing in detail depending on the terrain of the disease in which it developed

Rigdon (194-) investigated the cause of death in a child infected with *P falciparum* and reported that there was evidence indicating that shock associated with anoxaemia was responsible A similar suggestion was put forward by Kean and Smith (1944) who reported that 22 of 100 fatal cases of *P falciparum* infections examined by them died from shock or related phenomena Kean and Taylor (1946) have recently described six further cases of *P falciparum* infections which developed symptoms of shock during the clinical activity of the disease

Although the clinical picture drawn by the various authors who have described algid and similar forms of malaria is uniformly one of vascular collapse certain criteria are required before the syndrome can be accepted as one of shock The most important of these are a falling blood pressure and loss of circulating blood volume

The fall in blood pressure has been observed by practically all workers who have measured it For instance Kean and Taylor report the appearance of a shock-like state in an adult patient during the course of a P *falciparum* infection in which the systolic pressure fell to 64 mm Hg and the diastolic pressure was too low to measure The pulse rate at this stage was 155 and respiration rate 40 In another case the blood pressure fell from 95/65 on admission to 60/40 six hours later when

algid symptoms appeared and rose again to 85/56 five hours later when intravenous saline was administered

There is little available information regarding circulating blood volume changes in malaria but what evidence there is indicates that there is a substantial reduction during the algid syndrome Feldman and Murphy (1945) investigated the changes in blood volume and erythrocyte concentration in untreated *P vivax* and *P falciparum* infections They found that in the active phase of the uncomplicated disease there was a uniform increase in plasma volume (which they considered to be a reaction to compensate for the loss of erythrocytes) whereas the total blood volume was lower in the paroxysm than in the apyrexial intervals One patient with severe *P falciparum* infection developed a state of shock in which the blood pressure was low the skin cool and moist and the pulse rapid and almost imperceptible The venous haematocrit reading was found to be lower than the control value taken before shock developed Nevertheless the plasma volume was nearly 50 per cent below the control value and the total blood volume nearly 60 per cent below Hence in this case there was considerable reduction in the blood volume which did not show in the haematocrit estimations because of the coincident destruction of red cells

Kean and Taylor studied the blood volume in three of their cases by means of repeated haematocrit and haemoglobin determinations and red cell counts and found evidence of considerable reduction in all In the case referred to above in which the diastolic blood pressure was too small to record the haemoglobin concentration rose to 114 from a previous figure of 80 per cent and the erythrocyte count to 5 08 from 4 35 million per cu mm The haematocrit reading during the algid phase was 57 per cent Fifteen hours later after intravenous administration of saline and clinical recovery the haemoglobin concentration was 80 per cent the red cell count 4 05 million per cu mm and the haematocrit 37 per cent There was thus clear evidence in this case of haemoconcentration and therefore of loss of blood volume during the collapse Similar but less complete results were obtained in the other cases

More observations of this sort are required but there is little doubt that in the cases examined by Feldman and Murphy and by Kean and Taylor the presenting syndrome was one of medical shock The clinical features blood pressure changes and pathological findings of many other cases recorded in the literature indicate that this condition of medical shock occurs frequently in complicated malaria

Local circulatory disturbances

The general circulatory effects mentioned above influence the circulation through the tissues of the body but there are certain local conditions which modify their effects in some organs Many of these phenomena have already been discussed in the chapters devoted to the various organs Examples are the shunting of the renal flow from the cortex to the medulla and the impedance of the escape of hepatic venous blood from the liver These factors are influenced by nervous reflexes which may be set off by the general or local responses to malaria infection

The anatomical make up of the individual organ also determines to some extent the rate of blood flow through it Thus in the spleen the existence of an open circulation in the loose tissue of the Billroth cords slows the blood flow considerably The alleged spincter-like properties of the efferent extremities of the splenic sinuses described by Knisely would have a similar effect The sinuses of the liver and bone marrow are also believed to slow the circulation in those organs but in the former direct observation indicates that there is normally a rapid although to some extent intermittent flow through the lobules (Wakim and Mann 1942)

Mechanical obstruction to flow is also evident in some organs in malaria Changes in the vessel walls including swelling and occasionally phagocytic activity of the endothelial cells and in the late stages of the disease stickiness of leucocytes cause some slowing of the blood flow Obstruction to vessels in areas in which collateral circulation is poor such as the heart muscle and parts of the brain also occurs occasionally as a result of accumulation of parasitized cells free parasites and debris Free malarial pigment is also sometimes present in sufficient amounts to interfere with blood flow True thrombosis and agglutination of red cells especially in the form of sludge may also be concerned in the late stages of the disease All of these are however of minor importance The principal factor involved apart from local specific circulatory reflex changes such as those appearing in the kidney is the development of the physiological condition of stasis which is fundamentally reversible in nature and arises from the local loss of fluid from the circulating blood This phenomenon develops primarily in those organs in which the capillaries and small vessels are normally impermeable to protein and is not seen in other organs in which the vessels may be normally permeable Thus stasis is prominent in the brain the heart and sometimes in the

gut but is practically never seen in the liver the kidneys or the bone marrow

It has been pointed out that the circulation in a given organ is influenced considerably by the general circulatory changes which occur during the disease It is equally true to say that the local changes in blood flow in some organs may affect the general circulation For instance ischaemia of the adrenals results in the outpouring of adrenalin and so causes general vasoconstriction This had been discussed in detail elsewhere but it serves here to illustrate the complex nature of the ultimate circulatory response to the malaria infection

The initial circulatory phenomena of the paroxysm can be explained in terms of the effects of some form of stimulation (and possibly associated inhibition) of the vasomotor centres particularly those of the hypothalamus As the disease progresses however particularly if as in *P falciparum* infections a more or less constant fever supervenes other factors influence the circulation and may lead ultimately to complete vascular collapse in which the circulation fails not because of cardiac failure but because of loss of circulating blood volume and reduction in venous return to the heart

The depletion of blood volume is enhanced in many serious cases by direct loss of fluid in the form of vomit bowel motions and sweating but loss of fluid in this manner cannot account for the majority of cases The degree of parasitaemia is not necessarily the deciding factor either since shock often appears in cases in which there is only moderately severe parasitaemia and may not develop in heavy infections Kean and Taylor for instance mention a case with 20 per cent invasion of erythrocytes in which there was no evidence of shock whereas shock appeared in others with much lower parasitaemia Anaemia in these cases was not in itself the precipitating factor For instance in one patient the erythrocyte count before the onset of shock was 4 35 million cells per cu mm and 4 65 million per cu mm after recovery

It seems therefore that there must be some factor at work which allows progressive loss of plasma fluid from the circulating blood and that the tissue at fault must be the endothelium of the smaller blood vessels *That this is so is indicated by the development of stasis* in certain organs notably the brain

The initial cause of damage to the endothelium is not clear There is however a general factor of very great importance which may not perhaps be the initiating factor but which must greatly influence the development of the changes in the endothelium *This is the production*

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of tissue anoxia Some degree of anoxia must potentially exist in all cases of malaria since the destruction of erythrocytes the conversion of haemoglobin into haemozoin and the removal of oxygen from oxyhaemoglobin by the parasite all work together to produce anoxaemia the degree of which will in the first instance depend on the degree of parasitization of the erythrocytes and the duration of the illness

It is moreover possible that histotoxic factors arising directly or indirectly from the parasitic invasion may influence the dissociation of the haemoglobin of parasitized red cells and also the power of the tissues including the endothelium to use the oxygen with which they are supplied Evidence for such interference with dissociation is lacking but there is a striking similarity between the tissue changes produced by artificially induced histotoxic anoxia e g that of barbiturate poisoning and those of malaria (Hartman 1938)

In the tissues the reduction of blood flow brought about by the various mechanisms described above leads to the production of stagnant anoxia and this in turn to exacerbation of the injury to the endothelium and a further change in permeability and increased loss of protein and fluid In tissue such as the brain the process is progressive and eventually the cells form masses which may obstruct the flow The tissues now become completely anoxic and the degenerative changes which were in evidence from the beginning of the anoxia pass on to necrosis The pattern of the lesions formed is dependent on the circulatory arrangements of the organ as explained elsewhere but the basis of all of them appears to be anoxia of one form or another the noxious effects of which include an increase in the permeability of vascular endothelium to protein resulting in the extravascular escape of plasma fluid This effect of anoxia on the small blood vessels has been demonstrated experimentally by Landis (1927) who investigated the effects of various local conditions on the permeability of the endothelial walls of the vessels of a frog s mesentery He found that accumulation of carbon dioxide and acid products of metabolism within the physiological range produced no measurable changes in fluid movement from the lumen to the extravascular tissues Marked changes were however produced by lack of oxygen which alone was found to alter the permeability sufficiently to allow the passage of protein across the vessel walls The effect of oxygen lack was reversible Stasis which developed during oxygen deficiency could be resolved by bathing the tissue in oxygenated Ringer s solution Similar stasis was observed by Florey (1926) in isolated capillaries in the exposed mammalian omen-

tum The vessels appeared as if all the fluid content had been filtered off leaving a solid mass of corpuscles within the lumen Spontaneous resolution sometimes took place These changes occurred without measurable change in the diameter of the vessels

Landis found that the permeability of capillaries was increased during the period of stasis and rapidly returned to normal after resolution He came to the conclusion that local asphyxia could alone account for the phenomena of stasis and changed permeability resolution of the stasis being an indication of restoration of oxygenation and return of normal permeability to the vessel wall Oxygen lack in addition to its indirect effects produced by the accumulation of tissue metabolites exerted a direct action on the vessel wall increasing its permeability to fluid and protein The asphyxiated vessel wall acted purely as a passive filter the movement of fluid across it depending upon the capillary blood pressure and effective osmotic pressure of the plasma protein In severe asphyxia the cells allowed protein to escape and the osmotic pressure fell Exposure to complete oxygen deprivation for three minutes resulted in an escape of fluid at four times the normal rate and a reduction of osmotic pressure by one half

The initiating factor

Increase in endothelial permeability to protein with associated loss of fluid stasis of red cells and diapedesis of red cells through the vessel walls occur in other forms of anoxia besides asphyxia These phenomena are also characteristic of acute inflammation resulting from infective agents or trauma In the former case the phenomena are primarily induced by the presence of the agent and its products which in certain instances have been found to include polypeptides specifically active in increasing endothelial permeability (Chain and Duthie 1939) The circulatory phenomena of malaria are in many ways so similar to those of acute general inflammation (Cannon 1941) that it would not be surprising to find that similar substances were produced during malaria infections and acted as the factors initiating the vascular damage

The vascular changes of malaria are generalized although they may be manifested differently in the various organs depending on the anatomical and physiological type of microcirculation present There is ample evidence of damage to the vascular endothelium and indirect evidence in some organs of local and sometimes general fluid loss from the vessels resulting in retardation or obstruction to blood flow from stasis In organs such as the brain in which the vessels are

normally impermeable to protein these latter factors predominate In others such as the liver where the vessels are normally relatively permeable and free escape of protein and fluid to the tissues is physiological they are not much in evidence

The vascular phenomena of malaria are however so widespread through the tissues that some kind of common initiating factor must be postulated So far we do not know what this is The identification of some substance in malaria capable of initiating generalized leakage of fluid from the blood vessels would make the whole conception of the disease more intelligible There is however no information concerning the action of plasmodia or their products on the permeability of the vascular endothelial wall

The existence of a soluble malarial toxin has often been postulated in order to explain the pathogenesis of malaria but as was pointed out when referring to the paroxysm attempts to demonstrate its existence have so far failed Metabolites of the parasite and those of the damaged tissue may exert a local effect but are unlikely to initiate a general reaction Local changes in certain organs such as the brain and adrenals probably initiate physiological responses throughout the body which can explain many of the humoral phenomena but these reactions do no more than modify the progress of the disease It has been frequently suggested that the parasitic pigment may be the basic factor concerned in tissue damage but this has not been proved and seems on the available evidence to be unlikely The fate of blood pigments released during intravascular haemolysis is discussed elsewhere (Chapter IV) The possibility that these pigments may exert toxic effects on the tissues is remote

The factor common to all the tissue changes of malaria thus appears to be a vascular one (Birks 1943) In some organs the circulation is slowed because of obstruction to flow arising from stasis or near-stasis and sometimes from thrombosis In others e g the liver and the kidney it is also affected by physiological reflex vascular responses In all organs tissue damage appears to arise in the final analysis from anoxia due to a combination of local and general circulatory disturbances generalized anoxaemia arising from changes in the erythrocytes and their destruction and histotoxic effects of metabolites and possibly toxins

We can only guess at the nature of the initiating factor in this complicated chain of events The information available suggests that it may be some non-specific substance like the polypeptides of inflammation which exerts its effect upon the vascular endothelium

probably by producing a state of histotoxic anoxia and allows local escape of protein and fluid leading to stasis in some organs and ultimately to generalized loss of blood volume and circulatory derangements It may be that this substance may also affect the cerebral centres and give rise to the paroxysms

FACTORS MODIFYING TISSUE RESPONSE

Host-parasite reactions

So far we have discussed mainly the reactions of the host to the parasitic invasion but the progress of malaria is often also considerably modified by the effects of the host upon the parasite The appearance of synchronicity of maturation of broods of parasites the alteration of the asexual cycle by variation of the habits and environment of the host and the limitation of multiplication of *P vivax* resulting from differential invasion of young red blood cells are examples of the latter Fever also affects the parasites as was demonstrated by Plotner who concluded from his experiments that a short period of normal temperature in the host was necessary for the proper development of *P vivax* The most important factors influencing the host-parasite reaction and hence the tissue responses are however the state of nutrition of the host during the invasion and the immunity reactions evoked

Nutritional status of host

It is a common clinical finding that malnutrition leads to increased severity of infection and there is now a considerable volume of evidence indicating that the growth and development of the parasite are dependent on the general nutritional state of the host and the presence *in vivo* (and *in vitro*) of certain essential substances such as biotin and pantothenate These matters are referred to elsewhere and need not be considered here (see Chapters II and VII)

Immunity

The immune reactions of the host to the malarial invasion are of interest here only in so far as they affect the general pathological picture The epidemiological features of immunity are outside the scope of this book and need be no more than summarized in order to provide a basis for the discussion of the more relevant immunological processes which influence the development of the parasite and the pathological and other changes arising in the host tissues

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to the disease Acquired immunity enables the host to resist reinfection with a strain of parasite with which it has been previously infected When a state of immunity exists in which there is a continued infection without apparent clinical response the condition is usually called tolerance When tolerance is associated with resistance to super-infection with the same parasitic strain the condition is one of premunition Some authors as will be seen below believe that immunity in human malaria is dependent as in bird malaria upon the persistence of a latent infection in the host This hypothesis has not been proved On the other hand there is ample evidence that humoral immune bodies are developed during a malarial attack and it has been shown that immunity may persist for a long time after the termination of the infection

There is no need here to discuss the arguments for or against the view that acquired immunity against malaria is dependent on continued infection Whether acquired resistance is called tolerance premunition or immunity is to some extent irrelevant since it appears to act in the same way whether residual infection is present or not i e by stimulating phagocytosis and destroying the parasites and not by interfering with their division

Non-indigenous individuals suffer most severely from malaria in the first few years of residence in an endemic area and thereafter become progressively less subject to severe attacks In indigenous populations the active disease is common in very young children but is rare in adults

The splenic index is used as a measure of the development of acquired resistance in a community (Daniels 1901 Christophers 1924) In an endemic area this index rises sharply in early childhood reaching sometimes over 90 per cent by the third year and remaining high until about the tenth year After this it declines rapidly and reaches a steady level in adult life provided the infection rate is continuous Gill (1914) has shown that a constant splenic index in a population continuing over a period of years represents a constant immunity Where infection is interrupted for any considerable length of time the degree of immunity falls and epidemics of severe malaria may occur

In young children in endemic areas the enlargement of the spleen is accompanied by parasitaemia and clinical evidence of malarial infection In older children parasites are less common and symptoms fewer and in adults parasites are scanty and symptoms rare

Thomson (1933) points out that the development of a high degree of acquired resistance in a community depends on continuous transmis-

Natural immunity

Certain individuals appear naturally resistant to malarial infections They may live continuously for years in endemic or hyperendemic areas apparently without acquiring the disease and they may fail to become infected after inoculation with parasites Yorke and Macfie (1924) James (1926 1931) and others found that a proportion of inoculated individuals appeared refractory to infection Such subjects are considered to possess a high degree of natural immunity to the strain of parasite concerned Lourie (1938) defines such immunity as resistance to infection with which the individual animal is endowed before it comes into contact with the disease

It is not known whether natural immune reactions on the part of the host always lead to complete destruction of the infecting parasite Rudolf (1926) for instance recorded cases in which parasites were found in the peripheral blood shortly after inoculation although no symptoms of the disease subsequently developed Thomson (1933) suggested that there might therefore possibly be a latent infection in some of the apparently naturally immune subjects It seems likely however that in most cases the parasite is completely destroyed According to Taliaferro (1941) natural immunity to malarial infection is largely dependent on the non-specific parasiticidal action of the phagocytic reticuloendothelial cells of the host there is no important inhibition of the asexual division of the parasite

Individuals in whom the infection is completely resisted represent extreme examples of successful natural resistance Lourie (1938) has pointed out that the process is active even in overwhelming infections For example in *P cathemerium* infections in canaries 15 merozoites are produced by each dividing schizont every 24 hours If there were no resistance to the development of the parasites it would therefore multiply its numbers 15 times each day until the infection of erythrocytes was complete In fact the multiplication rate is only about five times in the day so that natural resistance must account for 10 of every 15 merozoites liberated In the canary infected with *P cathemerium* this high rate of destruction is insufficient and the infection develops not because there is *no* natural immunity but because there is not enough

Acquired immunity

Continuous exposure to malarial infection over many years leads to the development of some degree of acquired resistance or immunity

after adequate treatment in a matter of months in most human cases but occasionally it persists for long periods after the infection has been terminated Thus James (1933) and Boyd Stratman-Thomas and Kitchen (1936) demonstrated immunity two and three and a half years respectively after inoculation and James and Ciuca five years afterwards In Boyd s case subinoculations were negative It is difficult to reconcile such findings with the hypothesis that immunity in human malaria is dependent on the presence of a latent infection

Yorke and Macfie (1924) suggested that quinine if administered during a heavy infection would stimulate the production of immunity by suddenly liberating large amounts of antigen following the destruction of the parasites Sinton (1938) however held that the immune response is determined by the amount of antigen which can be effectively used by the body and that the appearance of an excess of antigen resulting from sudden rapid destruction of parasites would not be as effective as the same quantity of antigen acting over a longer period This view is more in keeping with the experimental evidence and it appears that the use of antimalarial drugs during the course of a malaria infection affects the production of immunity only in so far as it interferes with the development of the infection

The immediate reaction of the body to malarial invasion is the non-specific destruction of the parasites which according to Taliaferro is brought about mainly by phagocytosis the degree and success of which depend on the natural resistance of the host It is not known whether the parasites are killed before being phagocytosed but it is probable that they are engulfed in the living state The cells chiefly concerned with phagocytosis are the ordinary reticuloendothelial macrophages of the spleen and liver and to some extent of the bone marrow The phagocytes of other tissues are relatively unimportant

It is intimate contact of the blood cells with the phagocytes that is responsible for the concentration of phagocytosis in the liver and spleen rather than any special dynamic feature of the circulation in these organs It has often been stated that the presence of sinusoidal vessels retards the blood flow but this is certainly not always the case in the liver in which the circulation is apparently normally fast (Wakim and Mann 1942)

Once the disease is established in the host the natural resistance becomes reinforced by the development of acquired immunity which gives rise to a specific increase in phagocytosis confined mainly to the macrophage cells of the reticuloendothelial system and especially prominent in the spleen and to a lesser degree in the liver In self-

sion of the infection He estimates that it requires about 15 years continuous exposure to infection to produce tolerance in an individual This length of time is required in naturally acquired infections because of the numerous strains of parasites to which the subjects are exposed

The introduction of malaria therapy for the treatment of general paresis by Wagner-Jauregg has enabled the development of acquired immunity to be studied under controlled conditions

Yorke and Macfie (1924) found that after a patient had recovered from an induced *P vivax* infection it was very difficult to infect him with the same strain of parasite This observation was confirmed by other workers who demonstrated further that the resistance to infection was highly specific Other strains of the same species as the initial strain produced modified clinical reactions but other species of parasites were unaffected and produced normal clinical reactions when inoculated Only one instance is known of resistance to one species being effective against infection by another species James and Ciuca (1938) found that patients resistant to strains of *P vivax* were also resistant to some extent to strains of *P knowlesi*

It has not yet been decided whether this resistance to infection with the homologous strain is due to latent infection or to a genuine humoral immunity In bird malaria the duration of immunity to infection is believed to depend on the existence of a latent infection since complete cure of the infection leads rapidly to loss of immunity On the analogy of bird malaria Thomson (1933) held that a similar latent infection was concerned in the maintenance of immunity to human malaria This has not however been proved experimentally and there is a good deal of evidence indicating that a genuine humoral immunity is developed in the absence of a latent infection

Immunity does not develop appreciably after a single infection in man unless the disease is allowed to progress for some considerable time Redmond (1941) states that about one year is necessary during which the patient should receive no treatment unless absolutely necessary If the disease is terminated early after infection very little immunity develops Repeated infections at short intervals lead to more rapid development of immunity For instance Ciuca *et al* (1934) found that 34 per cent of their patients showed resistance to *P vivax* after the first inoculation and 87 per cent after the third inoculation They obtained similar results with *P malariae* infections and Sinton (1940) showed a similar effect with *P ovale*

Effective immunity to a particular strain of plasmodium is lost

to Taliaferro the proliferation of macrophages is non specific and not directly associated with the development of acquired immunity

P knowlesi infection in *M mulatta* is normally a rapidly fatal disease In an animal in which the infection is adequately treated the changes in the spleen very closely resemble those seen in the milder self-limited simian infections already discussed The same active phagocytosis and proliferation of macrophages and lymphocytes is evident When however the disease is allowed to run its fatal course the histological picture in the spleen is complicated by a degenerative process which affects both the lymphocytic hyperplasia and the macrophages A tremendous loss of cells occurs (Taliaferro and Mulligan 1937) which masks the proliferative and phagocytic cellular activity which is present as Taliaferro has shown in the earlier stages of the infection

In human malaria phagocytosis has been described in almost every organ but there is general agreement that it is mainly concentrated in the spleen and the liver No regional concentration of phagocytosis such as that described in the spleen during the crisis of monkey malaria has however been reported in human infections Taliaferro suggests that this is due to the fact that most of the human tissue examined has been obtained from acute and often fulminating cases of *P falciparum* infection in which as in *P knowlesi* infections in *M mulatta* the degenerative processes of the later stages of the infection have been obliterated the phagocytic and proliferative cellular activity of the earlier stages

In the early stage of a malaria infection the phagocytosis associated with the natural immunity of the host is non-specific and the phagocytic cells ingest red cells parasitized red cells free parasites and pigment indiscriminately When acquired immunity has appeared the phagocytes although presumably still retaining their normal function as scavengers for other material become specifically active against red cells *parasitized red cells and free parasites*

There has in the past been a good deal of controversy over the ability of macrophages to ingest undamaged parasites or parasitized cells but it is well established now that they can do so particularly once some degree of acquired immunity has been achieved Mannaberg (1894) demonstrated the ingestion of free parasites by phagocytes in fresh preparations Thomson (1933) described the ingestion of apparently normal parasites by macrophages and polymorphs in human malaria both in the peripheral blood and in splenic smears The Taliaferros (19 9 1934) found that infected red cells were phagocytosed only slowly in normal animals but were engulfed vigorously after

limited or drug-controlled infections in animals it is possible to observe this specific cellular reaction but in human malaria it is frequently masked by concomitant degenerative processes The cellular reactions associated with the development of acquired resistance have been described most fully in bird and monkey malaria by Taliaferro and his colleagues (Cannon and Taliaferro 1931 Taliaferro and Cannon 1936 Taliaferro and Mulligan 1937 Taliaferro 1941) Their findings in Panamanian monkeys infected with *P brasilianum* illustrate the processes clearly In the early stages of a primary infection the free merozoites and intracorpuscular parasites are phagocytosed sluggishly by the macrophages of the spleen liver and bone marrow This response is the result of the host s natural resistance and is insufficient to prevent the development of the infection In the course of a few days after the appearance of the parasites in the blood the degree of parasitaemia increases to a maximum and then falls rapidly as the infection becomes subdued The period of maximum parasitaemia and the subsequent sudden reduction is referred to as the crisis of the infection and it is in this stage that the cellular phenomena of acquired resistance first become evident At the crisis masses of parasitized red cells accumulate regionally in the spleen and appear to be held in the Billroth cords The venous sinuses remaining relatively free In the course of a few hours intense phagocytic activity develops in the macrophages and the parasitized cells become avidly engulfed At the same time the phagocytic activity of the individual macrophages of the liver and marrow is also increased The macrophages of these three organs but particularly those of the spleen rapidly become engorged with parasitized erythrocytes parasites in all stages of development pigment and cellular debris The infection now becomes rapidly subdued and the critical fall in parasitaemia takes place In splenectomized animals the macrophages of the liver and bone marrow become the principal agents in reducing the infection Once the specific phagocytic activity of the macrophages has been established it reappears immediately on superinfection with the homologous strain of parasite The ingested cells and parasites disappear rapidly from the macrophages but the malarial pigment is retained for months

The appearance of specific phagocytosis in the spleen liver and bone marrow is associated with a local increase in the number of macrophages In the spleen Taliaferro believes that the new macrophages come mostly from the lymphoid tissue which shows a marked hyperplasia but the reticular cells of the stroma of both the spleen and the bone marrow also proliferate and give rise to macrophages According

cytosis possibly as the result of the existence in immune animals of some humoral agent equivalent to the opsonins of bacterial infections

Coggeshall (1941 1943) has recently reviewed the information available on the development of humoral immunity in malaria There is some evidence that neutralizing antibodies are formed during the infection in both man and monkeys Complement fixing and agglutinating antibodies are elaborated and possibly also precipitins The production of opsonins has not been demonstrated but Coggeshall believes that the specific agglutination of *P knowlesi* parasites by homologous immune serum is presumptive evidence that the parasites are sensitized *in vivo* and thus rendered highly susceptible to phagocytosis Circulating antibodies first appear in a primary infection in simian malaria in the immediate post-crisis period In human malaria the complement fixing antibody appears about three weeks after the onset of the attack and persists for months after the disappearance of the parasites

Immune bodies probably affect the development of pathological changes in malaria only indirectly by influencing the processes of phagocytosis and possibly by the agglutination of erythrocytes and consequent interference with the circulation through the small vessels of the body

Autoantigen-antibody reactions

Gear (1946) has however recently pointed out the possibility of the existence of another process which may be responsible for some of the pathological changes seen in malaria He has suggested that the parasitic invasion of the erythrocytes alters their chemical constituents and gives rise to autoantigens which are capable of stimulating the production of autoantibodies in the form of autohaemolysins These antibodies in the presence of red cells and complement cause haemolysis This is an attractive theory in many ways and has a parallel in bacterial diseases in which it has been shown that a mixture of organ tissue plus streptococcal or staphylococcal extracts becomes autoantigenic and gives rise to autoantibodies which react with the homologous tissues It is tempting to apply the analogy further and suggest that the lesions of the organs e g in the cells of the kidney tubules and liver lobules developing in malaria may arise to some extent from such antibody-antigen reactions the autoantigen in this case being created as a result of tissue change arising indirectly from the malarial invasion either as a result of the activity of parasitic products or of the tissue anoxia which develops

injection into animals which had acquired immunity Active ingestion of red cells parasitized red cells and free parasites in enormous numbers was further demonstrated by Taliaferro and his colleagues as described previously, during the crisis of self-limited malarial infection in monkeys and birds There is however no direct evidence that the parasites are alive when ingested and as Taliaferro and Mulligan point out the development of specific phagocytosis does not exclude the possibility of the existence of other parasiticidal mechanisms such as lysins circulating in the plasma

The increased phagocytosis of acquired immunity involves both parasitized and unparasitized erythrocytes both of which are ingested in very large numbers Fairley (1933) has therefore suggested that it may be directed in the first instance against the erythrocytes rather than the parasites There is some evidence to support this view Brown and Broom (1935) for instance have demonstrated an equal reduction of surface electrical change on both parasitized and unparasitized red cells which appears at the crisis of the infection in bird malaria They found this reduction in change was non-specific and suggested it was related to the appearance of immune bodies in the plasma and associated changes in plasma protein They considered that a reduction in surface change rendered an erythrocyte more susceptible to phagocytosis Knisely (1942 1945) observed the precipitation of crystals around and about erythrocytes in the circulation of monkeys in the late stages of *P knowlesi* infection and reported that this precipitate made the erythrocytes sticky towards phagocytes which readily ingested them

It thus appears that in the crisis of infection in bird malaria and in the late stages of monkey malaria all erythrocytes whether parasitized or not become more susceptible to phagocytosis Some of the avid ingestion of red cells observed in malaria may therefore result from similar changes in human malaria However Taliaferro and his colleagues have shown that there is in addition a more specific phagocytic reaction which is directed against the parasites and parasitized erythrocytes For instance they have shown that the intense activity of the macrophages in the spleen is stimulated at once by attempted superinfection at a time when it is unlikely that any general alteration in erythrocytes can have taken place and when Knisely s phenomenon is absent Moreover the Taliaferros found that erythrocytes from an infected animal were phagocytosed slowly when injected into a normal animal but were ingested extremely rapidly in an animal possessing acquired immunity (in association with latent infection) They therefore suggest that infected cells must be specifically susceptible to phago-

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SYNTHESIS THE PATHOGENESIS OF MALARIA

The account of malaria given above includes a description of the lesions in most of the important organs except the lungs and gastro-intestinal tract Reference to the literature will show that in the latter organs the changes are primarily of the same nature (Spitz 1946)

Certain pathological processes appear to be fundamental to the development of the lesions in all organs It should be noted here that selective localization of parasites is not an essential factor since apart from accumulations in the liver and spleen concentration of parasitized cells is not usually evident in a particular organ even when the clinical symptoms indicate its specific involvement

The basic processes which determine the host s reaction to the parasite include (i) generalized anoxaemia (ii) damage to the endothelial cells of the vessel walls and (iii) general and local changes in blood flow which result in tissue anoxia

Anoxaemia arises primarily from the destruction of erythrocytes and consequent anaemia Parasitized cells are disrupted at sporulation and unparasitized cells by lytic processes of uncertain origin The degree of anoxaemia is enhanced by interference with the oxygen carrying capacity of erythrocytes due to conversion of haemoglobin into haemozoin and removal by the parasite of oxygen from the oxyhaemoglobin and in many cases by incomplete oxygenation resulting from pulmonary involvement Damage to the vascular endothelium is a common histological finding in cases of severe malaria and is indicated in the pathological picture by the development of stasis (which depends on local escape of plasma fluid) in certain organs notably the brain and heart Changes in blood flow in their extreme form amount to medical shock In less severe cases the circulatory disturbances are the result of the prevailing fever and local vascular changes involving redistribution of blood flow in certain organs particularly the liver and kidney Direct mechanical obstruction to blood flow through small vessels also occurs especially in those organs in which stasis is prevalent and may become exaggerated in the late stages of the disease by complicating factors such as agglutination of erythrocytes and the formation of sludge or swelling and shedding of the lining cells of the sinuses for example in the liver The humoral effects of local tissue ischaemia e g in the adrenal probably influence both local and general vascular responses

As the disease develops the combination of anoxaemia endothelial damage and changes in blood flow leads to a general state of tissue

anoxia the effects of which are enhanced by complicating factors such as immune reactions and autoantigens and possibly the direct action of histotoxic agents

The changes developing in tissues are at first reversible but ultimately become irreversible and go on to degeneration and necrosis The pattern of the tissue damage depends on local vascular responses and associated changes in blood flow and the effects of alterations in endothelial permeability For example in the brain stasis is the significant factor whereas in the liver and kidney it is rare and local circulatory re-arrangements predominate

The paroxysm accelerates the processes outlined above by raising the metabolic rate increasing the erythrocyte loss and disturbing the general circulation As we have seen the paroxysm is essentially similar to the febrile response to non-specific pyrogenic agents and can best be explained by the release at sporulation of some factor which initiates the vascular changes and possibly also exacerbates the endothelial damage arising from anoxia

The anoxaemia created by the loss of red cells and changes in pulmonary circulation is probably in itself insufficient in most cases of malaria to initiate the tissue changes and the host s response to the invasion There must be other factors involved about which at present there is very little information No specific toxin has ever been identified but the evidence in favour of the existence of some circulating diffusable agent is very strong The similarity between malaria and general inflammation is striking enough to suggest that some active agent e g a polypeptide fraction similar to that identified by Chain and Duthie may be liberated as a result of the parasitic invasion and act as in acute inflammation especially upon the vascular endothelium The phenomena of the paroxysm could similarly be accounted for by the presence of some diffusable substance liberated at sporulation and affecting the hypothalamic centres It is conceivable that these factors are identical and that the agent giving rise to the febrile phenomena of the paroxysm may also initiate the vascular damage and circulatory changes setting off the chain of reactions which become synthesized into the disease entity we call malaria

Whatever the answer may be it is clear that the general and local circulatory changes and the endothelial damage are of fundamental importance in the general development of the disease and that the local tissue damage is governed essentially by the development of anoxia arising from generalized anoxaemia the circulatory changes alteration in endothelial permeability and possibly also from histotoxic effects

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Chapter II

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CHAPTER III

THE BLOOD CELLS

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CHAPTER IV

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CHAPTERS VII AND VIII

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